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REVIEW ARTICLE

Evaluation of IL-17A and IL-17F levels in patients with acne vulgaris and their association with disease severity.

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Abstract:

Background: Acne vulgaris is a very common, long-lasting, inflammatory skin condition that mostly affects the pilosebaceous area on the face, neck, upper trunk, and back. It is a widespread, multifactorial skin condition that affects people all over the world. The pilosebaceous follicle of acne vulgaris causes both non-inflammatory and inflammatory lesions. The natural cycling of sebaceous follicles can be disrupted by environmental and genetic variables, which may lead to the improvement of inflammatory injuries and comedones. The improvement of spot injuries is significantly influenced by genetic factors and pro-inflammatory cytokines. Aim: This study aims to evaluate IL-17A and IL-17 F levels in adults with acne vulgaris and their relationship with acne strictness. Methods: This study included 60 patients with acne vulgaris who were admitted to the hospital from November 2022 – March 2023, and another group consisted of 60 healthy individuals used as a control. An immunological study was conducted to evaluate serum IL17A, and IL17F using the Sandwich ELISA technique. Results: Both Il17A and IL17 F were significantly higher in patients with acne vulgaris.

Conclusion: Both IL17A and IL17F, can be used as predictive and diagnostic biomarkers for acne vulgaris.

Keyword: Acne vulgaris, IL17A, IL17F.Inflammatory and non-inflammatory lesions of the skin, pilosebaceous unit.

Introduction

Acne vulgaris is a very common, chronic, inflammatory skin condition that mostly affects the pilosebaceous area on the face, neck, upper trunk, and back. Heng and Chew [1]. Acne vulgaris appears most frequently in areas of the body with a high concentration of sebaceous glands, for instance the face and to a lesser extent the trunk, where sebaceous follicles form the majority. The neck and proximal superior extremities can likewise be influenced at times. The pathognomonic comedo, a congested follicle that can be locked or open, marks the beginning of the disease [2]. The natural cycling of the sebaceous follicles can be disrupted by environmental and genetic variables, which may lead to the development of comedones and inflammatory lesions. The improvement of acne injuries is significantly influenced by genetic factors and pro-inflammatory cytokines [3]. Numerous factors contribute to acne, including excessive sebum production (hyperseborrhea), changes in sebum composition, elevated Propionibacterium acnes proliferation, hyper-keratinization of the skin's pilosebaceous units, inflammatory reactions, immune system disorganization, and hormonal dysregulation [4]. There are six members of the interleukin-"17 family (IL-17A to IL-17F"), and all of them with their associated receptors have recently been discovered. IL17 release from activated T cells and has a crucial role in the pathogenesis of various skin diseases[5]. This family primarily affects the host's defensive systems against foreign antigens by triggering cytokines and chemokines, drawing in neutrophils, triggering the production of antimicrobial proteins, and altering T-helper cell differentiation [6]. Important members of the IL-17 family of cytokines, which controls both innate and adaptive immunity, include L-17A and IL-17F. L-17A has been linked to autoimmune and chronic inflammatory disorders[7]. IL-17F, identified in the year 2001, shares the same chromosomal location as IL-17A, specifically at the 6p12 locus on chromosome 6 [8]. IL-17A, also known as IL-17, is the initial and most extensively researched cytokine in the IL-17 family. It is produced not only by Th17 cells, but also by various other cell types including γδ T cells, NKT cells, NK cells, neutrophils, and eosinophils. IL-17A, IL-17F, and the IL-17A/F heterodimer have been associated with inflammatory responses and protecting the host against infections. When IL-17A, IL-17F, or IL-17A/F activate the IL-17RA signaling pathways, they induce the production of proteins with pro-inflammatory and antimicrobial properties. These proteins play a crucial role in neutrophil recruitment, infection control, and, when overproduced, can induce tissue death. IL-17A, IL-17F, and IL-17A/F are known to trigger the production of various cytokines (like IL-6, G-CSF, and GM-CSF), chemokines (such as CXCL1, CXCL2, and CXCL8), matrix metalloproteinases

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(MMP1, MMP3, and MMP9), and antimicrobial peptides (like β defensin-2, S100A7, and S100A8) from a range of cell types [9]. IL17 plays a pivotal role in the differentiation and growth of keratinocytes. Lai, et al. [10] demonstrated that the binding of IL17 to its receptor in keratinocytes induces the expression of the regenerating islet-derived protein 3-alpha (REG3A). This protein, in turn, provides feedback to the keratinocytes, blocking the cessation of differentiation processes and promoting cell proliferation. This is achieved through its interaction with exostoses-like 3 (EXTL3), which subsequently leads to the activation of the phosphatidylinositol 3 kinase (PI3K).

Material and method:

Study design:

This case-control training stayed accompanied in Al-Diwaniya governorate. The study population consisted of 60 patients (24 men and 36 women) suffering from acne vulgaris, with ages ranging from 13 to 38 years. Additionally, 60 healthy individuals (28 males and 32 females) within the age range of 14 to 38 years stayed comprised as control subjects. The participants were recruited from the consultant clinic for dermatology at Al-Diwaniya Teaching Hospital, under the supervision of a dermatologist. Diagnosis of acne was made based on patient history, clinical examination, and data collected using a questionnaire. Information such as name, sex, age, disease severity, disease duration, family history, treatment, and site of acne were recorded for each case. It should be noted that patients who developed acne as a result of steroid drug usage were excluded from the study. For each participant, three milliliters of intravenous blood stayed collected using a throwaway syringe in an aseptic method. Three milliliters of the blood stayed placed in a gel tube and permitted to coagulate. The serum stayed detached via centrifugation at 1500 rpm for 5 minutes, collected in a plain tube, and stored at -20°C. This serum was later used for ELISA testing to conclude the IL-17 concentration.

laboratory work

Immunological study conducted to evaluate serum IL17A,IL17F using Sandwich ELISA technique. The serum level was determined using a commercial kit, which employs the Enzyme-linked immunosorbent assay (ELISA) technique. The wells of the plate come pre-coated with either a human IL-17A antibody or IL-17F antibody. Any IL-17A or IL-17F present in the sample, when added, will attach to the antibody-coated in the well. Then, a biotinylated human IL-17A or IL-17F antibody is introduced and will bind to IL-17A or IL-17F present in the sample. Following this, streptavidin-HRP is added, which binds to the biotinylated IL-17A or IL-17F antibody. After an incubation period, unbound streptavidin-HRP is removed during a wash step. A substrate solution is then added, and a color develops, the intensity of which is directly proportional to the quantity of human IL-17A present. The reaction is halted by the addition of an acidic stop solution, and the absorbance is measured at 450nm.

Results

Subjects Immunological Analysis Results:

Serum Interluekin-17A (IL17A) level in Acne Vulgaris patients as well as healthy control.

The comparison of serum Interleukin-17A (IL-17A) levels among acne vulgaris patients and subjects of healthy control has been accepted over the years, and the outcomes are presented in Table (1) and Figure (1). Mean ranks of serum IL-17A were 199.62 ± 56.39 ng/ml and 362.27 ± 137.77 ng/ml, in healthy control and acne vulgaris patients, respectively; the level stood highly important, higher than in group patients in contrast with healthy control issues ($P < 0.001$).

Evaluation of Interleukin-17A (IL-17A).

To assess the IL-17A cutoff value and its potential as a diagnostic or adjunctive diagnostic test for Acne Vulgaris, receiver operating characteristic (ROC) curve analysis was conducted. The results of this evaluation are presented in Table (2) and Figure (2).

Frequency distribution of IL-17A levels according to Family history.

The comparison of IL-17A levels according to family history stayed approved, and the outcomes were confirmed in Table (3). Mean ranks of serum IL-17A were 391.37 ± 155.33 ng/ml and 346.31 ± 126.99 ng/ml.

Frequency distribution of IL-17A levels according to severity.

The comparison of IL-17A levels according to severity of disease has been accepted out, and the outcomes stayed validated in table (4). Mean ranks of serum IL-17A were 267.94 ± 73.53 ng/ml, 343.63 ± 93.42 ng/ml, and 525.43 ± 124.75 ng/ml, in patients with ("mild," " moderate," and " severe") disease separately.

Serum Interluekin-17F (IL17F) level in patients with Acne Vulgaris and healthy control.

The comparison of serum interleukin-17F (IL-17F) level among acne vulgaris patients and subjects of healthy control has stayed approved, and the outcomes were confirmed in table (5) and figure (3).

Evaluation of Interluekin-17F (IL-17F).

In order to assess the IL-17F cutoff value and its potential as a diagnostic test or adjuvant diagnostic test for Acne Vulgaris, receiver operating characteristic (ROC) curve investigation was carried out. The outcomes of this analysis, including sensitivity, positive predictive value (PPV), specificity, negative predictive value (NPV), and the area under the curve (AUC), can be found in table (6) and figure(6).The determined IL-17F cutoff value was > 63.66-fold, with a sensitivity of 81.7%, specificity of 75.0%, PPV of 76.6%, NPV of 80.4%, and an AUC of 0.873(0.807-0.940).

Discussion

The result of the current study, as mentioned in table (1), clarifies that the mean levels of serum IL-17A were 362.27 ± 137.77 ng/ml and 199.62 ± 56.39 ng/ml in acne vulgaris patients and subjects of healthy control, respectively; the level stayed highly significantly higher than in the patients' group in comparison with the healthy control subjects (P < 0.001). The findings of the present study align with the outcomes of the research conducted by Abd-Elmaged, et al. [11], conducted in Egypt, involving 135 acne patients, who discovered that the average serum levels of IL-17, measured using ELISA assays, were 544.2 ± 477.4 SD in patients and 42.2 ± 8.1 SD in the control group, indicating a significantly higher IL-17 level in the patient group. Another study was carried out by Kelhälä, et al. [12], conducted in Finland, using a different method, which demonstrated that the enhanced production of Th17-related cytokines in acne lesions was verified at the protein level by cytokine profiling. This profiling was done using Luminex technology on manually crushed skin biopsies from German patients. Moreover, a significantly greater quantity of IL-17A-positive (IL-17A+) cells was detected by immunohistochemistry in both the papillary dermis and surrounding the sebaceous follicles. One explanation for the increase in IL17 was given as follows by Agak, et al. [13]: It has been observed that P. acnes can trigger the release of IL-17 and IFN-γ from CD4+ T cells in laboratory settings, and IL-17+ T cells were predominantly found in perifollicular infiltrates of inflammatory lesions. This supports the connection between acne and the Th17/IL-17 pathway. Proteins from Propionibacterium acnes, which are immunogenic, would be released in the follicle, processed by Langerhans cells, and then presented to CD4+ cells. The subsequent secretion of cytokines would stimulate the differentiation of naïve CD4+ T cells into Th-17 cells. These Th-17 cells would then produce IL-17, leading to inflammation, which is a key characteristic of acne vulgaris (AV). Another reason was proposed by Ebrahim, et al. [5].It was demonstrated that the serum level of IL-17 significantly rises with increasing disease severity. This could be due to an intensified inflammatory response triggered by Th17 cells. These findings are consistent with previous observations made by Kelhälä, et al. [12], Agak, et al. [13], and Kim, et al. [14]. On the other hand, Topan, et al. [15] found no significant difference between Acne Vulgaris (AV) patients and the control group, suggesting that IL-17 may not play a significant role in the pathogenesis of AV. As per Figure (2), the results indicated that the IL-17A cutoff value was > 247.17-fold, with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve of 91.7%, 87.3%, 86.7%, 91.2%, and 0.915 (0.857–0.973), respectively. These current findings suggest that IL-17A could serve as an excellent diagnostic marker. Furthermore, the study revealed that serum IL-17 could be utilized for early diagnosis of AV when its level surpasses the cutoff value. With high sensitivity, specificity, and accuracy rates, it may also serve as a prognostic marker for AV, considering the sensitivity and specificity of serum IL-17 values in patients. The findings of this study are in alignment with the work of Ebrahim, et al. [5]. Additionally, according to the family history table (3), the results comparing between Positive family times past and sick with Negative family times past; the mean levels stayed upper in Positive family patients times past in comparison with patients with Negative family times past, but the difference stayed non-significant (P = 0.283).

This study takes into consideration between Il 17A in acne vulgaris sufferers and severity as mentioned in table (4), demonstrating that the mean levels were higher in patients with severe disease in contrast to other groups, and the variance stayed significant (P = 0.001). This result agrees with the study achieved by Abd-Elmaged, et al. [11], Ebrahim, et al. [5], where the researchers found that there was a significant increase in the serum level of IL-17 with increased disease severity. This could potentially be due to a more pronounced inflammatory response induced by Th17 cells. Acne scarring is the result of tissue damage surrounding pilosebaceous units, either due to active lesions or appearing from the beginning of acne development until its regression. This can occur in both severe and mild forms of acne. It's estimated that between 43% and 95% of acne patients may experience scarring from acne. On the other hand, Topan, et al. [15] reported that there was no important relationship established between the strictness of AV injuries of the patients and their IL-17 ranks (p=0.256). The study as mentioned in table (5) conducted that the mean levels of serum IL-17F were 93.97 ± 35.56 ng/ml and 49.36 ± 19.90 ng/ml, in acne vulgaris patients and healthy control, respectively; the level was highly significantly higher than in

the patient's group in contrast to the healthy control group (P < 0.001). The relationship between IL-17F and acne vulgarities has not been studied yet all over the world. McGeachy, et al. [16] revealed that IL-17F stakes the greatest resemblances with IL-17A, in expressions of cellular foundations and purpose. IL-17F and IL-17A are co-expressed on related genes and are frequently co-produced by kind 17 cells; therefore, in this study, we searched to find if there is any relation between IL17 F and acne vulgaris; therefore, the results revealed that Il 17 F is highly significant. Other studies confirm that the part of IL-17F in skin illnesses has not so far been entirely surveyed. Watanabe, et al. [17] examined the purposeful part of IL-17F in usual human epidermal keratinocytes (NHEKs).The findings indicate that IL-17F plays a significant role in skin diseases, particularly in psoriasis. IL-17A and IL-17F are essential cytokines responsible for recruiting and activating neutrophils. They can affect various cell types, including keratinocytes, endothelial cells, monocytes, and fibroblasts. This leads to the production of pro-inflammatory mediators such as IL-6, TNF-α, IL-1β, PGE2, nitric oxide, matrix metalloproteinases, and chemokines [12].

Conclusion

The results of the current study clarify that the mean levels of serum IL-17A and IL-17F in acne vulgaris illnesses stayed highly significantly higher than in the patients' group in comparison with healthy control subjects. IL17A and IL17F, is recommended to be used as predictive and diagnostic biomarkers for acne vulgaris illnesses.

Acknowledgement

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Interest Conflicts

The authors states that there are no conflicting interests.

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Table (1): Serum IL17A level in healthy control and Acne Vulgaris patients.

	Patients n = 60	Healthy control n = 60	P
Interluekin-17A level (ng/ml)			
Mean± SD	362.27 ± 137.77	199.62 ± 56.39	< 0.001 † HS
Range	146.40- 736.39	80.0 – 281.97	

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

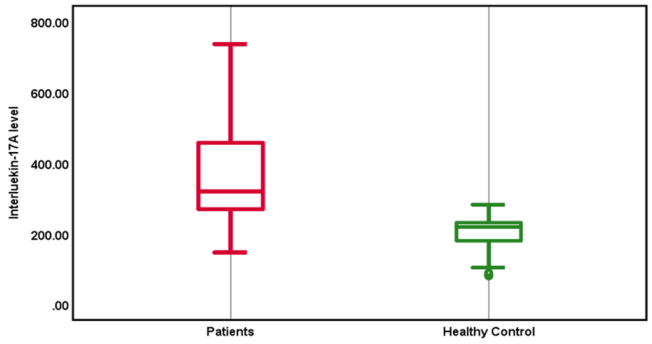

Figure (1): Serum IL17A level of patients and healthy controls.

Table (2): specificity and Sensitivity of IL-17A rank (> 247.17-fold) in Acne Vulgaris disease

IL-17A level	patients n = 60	Healthy control n = 60
> 247.17	55 (%)	8 (%)
> 247.17	5 (%)	52 (%)
Sensitivity %	91.7 %	
"Specificity" %	87.3%	
PPV %	86.7%	
NPV %	91.2%	
AUC (95% CI:):	0.915 (0.857- 0.973)	

CI: Confidence interval, AUC: Area under curve.

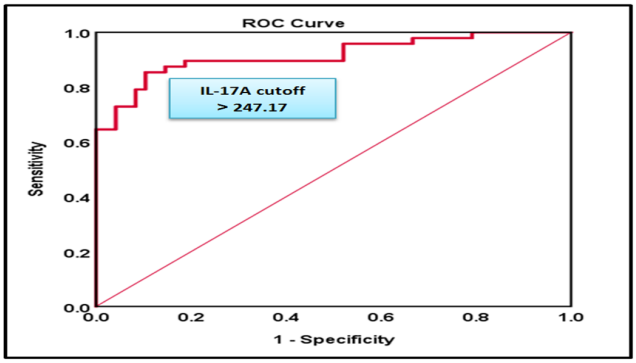

Figure (2) Receiver operative representative curve investigation of IL-17A for the design of likely analytic cutoff rate.

Table (3): Frequency distribution of IL-17A levels according to family history.

	Family history comparison		
	Positive n = 21	Negative n = 39	P
IL-17A			
Mean± SD	391.37 ± 155.33	346.31 ± 126.99	0.283 † NS
Range	146.40 – 736.39	214.71- 654.01	

n: number of cases; SD: standard deviation; †: independent samples t-test; NS: not significant at P > 0.05

Table (4): Frequency distribution of IL-17A levels according to severity of disease.

	Severity of disease			
	Mild n = 21	Moderate n = 24	Severe n = 15	P
IL-17A				
Mean± SD	267.94 ± 73.53	343.63 ± 93.42	525.43 ± 124.75	0.001 † S
Range	146.40 – 467.86	259.81- 654.01	334.91- 736.91	

n: number of cases; SD: standard deviation; †: independent samples t-test; S: significant at P > 0.001

Table (5): Serum IL17F level in patients with Acne Vulgaris and healthy control.

	Patients n = 60	Healthy control n = 60	P
Interluekin-17F level			
Mean± SD	93.97 ± 35.56	49.36 ± 19.90	< 0.001 † HS
Range	52.14- 199.95	5.38 – 89.75	

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

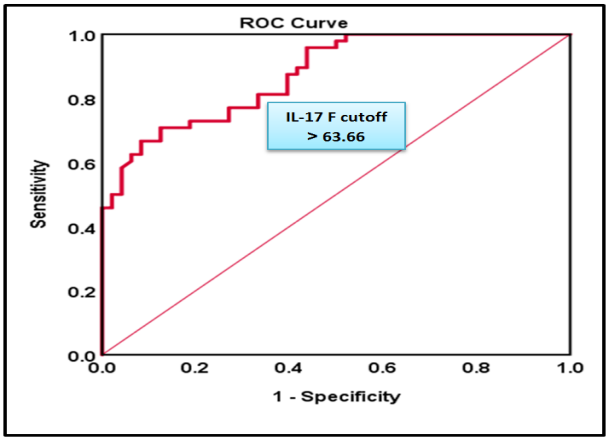

Figure (4): Receiver operative representative curve investigation of IL-17F for the design of probable analytic cutoff rate.

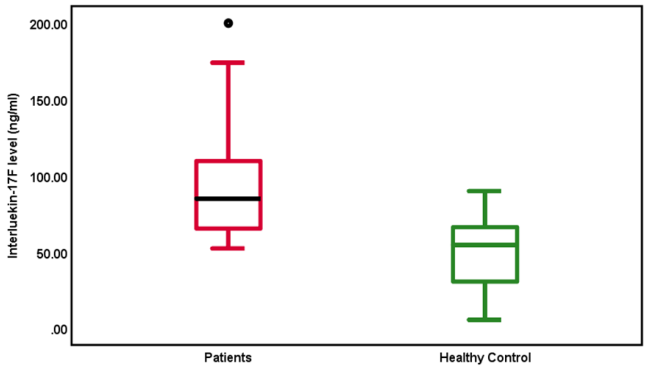

Figure (3): Serum IL-17F level of patients and healthy controls.

Table (6): specificity and Sensitivity of IL-17F rank (> 247.17-fold) in Acne Vulgaris disease

IL-17 F level	patients n = 60	Healthy control n = 60
> 63.66	49 (%)	15 (%)
> 63.66	11 (%)	45 (%)
Sensitivity %	81.7 %	
Specificity %	75.0%	
PPV %	76.6%	
NPV %	80.4%	
AUC (95% CI)	0.873 (0.807- 0.940)	

CI: Confidence interval, AUC: Area under curve.