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Evaluation of Interleukin-31 and C-reactive Protein in Inflammatory Acne Vulgaris Patients

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ABSTRACT

Background: Interleukin-31 (IL-31) is a cytokine that is commonly reported with inflammatory skin diseases and pruritus. It was positively correlated with disease activity in atopic dermatitis and primary cutaneous lymphoma. Epidermal keratinocytes express the subunits forming the receptor for this cytokine and thus may play a role in the inflammatory status of different dermatological diseases in which IL-31 might play a role.

Objective: This study aimed to evaluate the role of IL-31 and CRP in inflammatory acne vulgaris patients, for the understanding of a distressing disease and thus better therapies relevant for each case.

Patients and methods: This case-control study included 40 diagnosed patients with acne vulgaris and 40 healthy controls who were comparable in age and sex to cases. They were randomly chosen from the outpatient clinic of Mansoura University Hospital's Dermatology Department.

Results: There was a statistically significantly higher median IL-31 among the studied cases than in the control group (P=0.013). There was a statistically significant higher median CRP among studied cases than in the control group (P=0.035). Detection of CRP & IL-31 levels can be used as a reliable indicator in differentiating studied cases with the best-detected cut-off point being 0.650 &911 respectively yielding a sensitivity of 65 & 67.5 and specificity of 52.5% &50%, respectively.

Conclusion: This work suggested a pathogenic role of IL-31 in acne patients being related to the severity and inflammation, opening gates for a better understanding of the pathogenesis of common dermatological disease and thus better treatment results. Serum levels of CRP are elevated in acne vulgaris and are correlated with the disease severity.

Keywords: Acne Vulgaris, Interleukin -31, C-reactive protein.

INTRODUCTION

Acne is one of the most common skin diseases affecting adolescents and adults. It is a multifactorial disease manifested by comedones, papules, pustules, nodules, and scars [(1)].

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit and is among the most common dermatological conditions worldwide, with an estimated 650 million people affected. The pathogenesis of acne is known to be multifactorial which includes sebum overproduction, abnormal follicular keratinization, Propionibacterium acnes (P. acnes) proliferation, inflammation, and genetic factors [(2)]. The immune response plays role in inflammation observed in acne resulting from the recruitment of activated T lymphocytes [(3)].

Interleukin-31 (IL-31) is a cytokine that has been commonly reported with inflammatory skin diseases and pruritus. It was positively correlated with disease activity in atopic dermatitis and primary cutaneous lymphoma. Epidermal keratinocytes express the subunits forming the receptor for this cytokine and thus may play a role in the inflammatory status of different dermatological diseases in which IL-31 might play a role [(4)].

C-reactive protein (CRP) is one of the best indicators of systemic inflammation, considering that its serum levels show no circadian change across 24 h. Interleukin-1, Interleukin-6, and TNF-α which are found in the acne lesions are also major inducers of CRP production by the liver. Thus, CRP levels could be elevated in acne if the amount of local inflammation is high enough [(5)].

A usable and practical classification of Acne is important both in medical practice and research, it can be by using the description of the lesions using a scoring system offered by the European Guidelines Group which divides acne into four grades: comedonal, papulopustular, severe papulopustular, or severe nodular. Also disease severity was assessed using the Global Acne Score(GAGS). According to this scale, patients whose Global Acne Score was 1–18 had mild acne, those with a score of 19–30 had moderate acne, those with a score of 31–38 had severe acne, and those with a score greater than 39 had very severe acne [(6)]. Finally, itching was assessed using the analog scale (score from 0 to 10), where 0 indicated no itching and 10 was the worst itching ever [(7)].

This work aimed to evaluate the role of IL-31 and CRP in inflammatory acne vulgaris patients, for a better understanding of a distressing disease and thus better therapies relevant for each case, we hypothesize that IL-31 and CRP play significant roles in the pathogenesis of acne vulgaris, thus, we expect to see significant variations in their levels among different patient groups according to the grade of disease.

PATIENT AND METHODS

This prospective case-control study included 40 diagnosed patients with acne vulgaris and 40 healthy

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controls who were comparable in age and sex to the category of the cases. They chose at random from the outpatient clinic of Mansoura University Hospital's Dermatology Department.

Inclusion criteria: Both sex (males/females) with different types of inflammatory acne (papulopustular, severe papulopustular, or severe nodular). Patients aged above 14 years, and patients with no history of systemic therapies for at least 6 weeks before inclusion and no topical treatments for at least 2 weeks.

Exclusion criteria: Patients with pure non-inflammatory Comedonal acne. Pregnant patients. Patients with other systemic or dermatologic diseases or inflammatory disorders. Patients with systemic or topical drugs, and patients with body mass index BMI >25.

All participants (n=80) were divided into two groups:

Group A (n=40): forty patients with acne vulgaris of various degrees of severity, and **Group B (n=40)**: forty apparently healthy individuals of matched age and sex were chosen as a control group.

Typical clinical results were used to diagnose the patients. The following data were calculated for all patients and the control group: age, sex, family history, BMI, and disease duration for all patients and the control group. The magnitude of AV was also assessed using the GAG score.

The itching was assessed using the analog scale (score from 0 to 10), where 0 indicated no itching and 10 was the worst itching ever.

All patients have undergone serum levels of IL-31 concentration using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine ELISA Kits: R&D systems; ELISA reader 680 Bio-Rad).

Hypersensitive-C-reactive protein levels of the sera of case and control groups were measured using the sandwich ELISA method using standard calibration solutions provided by the manufacturer (Product Code: 3125–300, Monobind Inc., Lake Forest, CA 92630, USA).

Ethical consent:

Approval of the study was obtained from Mansoura University Academic and Ethical Committee. Every patient signed informed written consent for the acceptance of participation in the study. This work was carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi 2$) to calculate the difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare two independent groups of normally distributed variables (parametric data). P-value < 0.05 was considered significant.

RESULTS

Table (1) shows that there is no statistically significant difference between the studied groups as regards their age, sex, and body mass index with the mean age of the studied cases being 25 versus 23.3 among the control group. Seventy percent of cases are females versus 62.5% of the control group Mean body mass index of the studied cases is 24.55 versus 24.43 among the control group.

Table (1): Comparison of demographic characteristics of the studied groups

	Control group n=40	**Cases group n=40**	**P-value**
Age/years mean±SD	25.0±6.03	23.30±5.60	0.195
Sex N (%) Male Female	 15(37.5) 25(62.5)	 12(30) 28(70)	0.478
BMI (kg/m^2) mean±SD	24.43±3.15	24.55±1.78	0.828

Table (2) median age of disease onset among studied cases is 4 ranging from 2 to 16 years, median GAG score is 23 ranging from 11 to 52 and median itching scale is 2 ranging from 0 to 8.

Table (2): Onset of disease, GAG score, and itching score among studied cases

	Total number=40 Median (Min-Max)
Onset of disease/years	4(2-16)
GAG score	23(11-52)
Itching scale	2.0(0-8)

There was a statistically significantly high median IL-31 (ng/l) among the studied cases than in the control group (p-value 0.013*). There was a statistically significantly higher median CRP (mg/l) among studied cases than in the control group (p-value 0.035) **(Figures 1 & 2).**

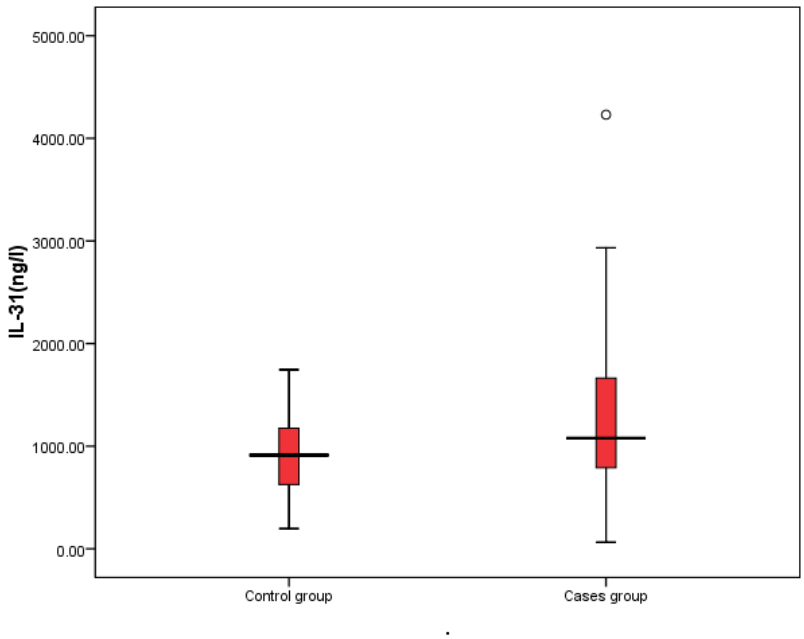

Figure (1): Comparison of IL-31 among studied groups

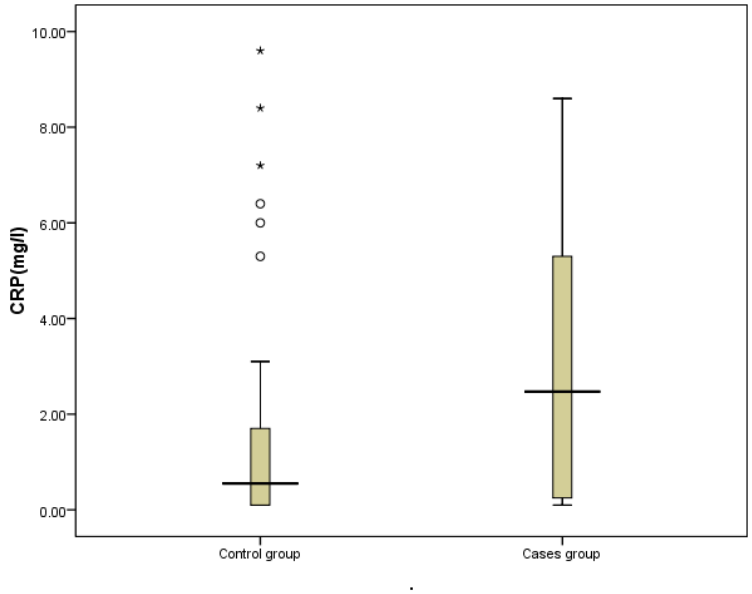

Figure (2): Comparison of CRP among studied groups

There was statistically significant correlation between CRP and the following; negative with BMI & onset of disease (r=-0.371 & -0.445) and positive with GAG degree & EGSS (r=0.356 & 0.343). However; IL-31(ng/l) shows statistically significant positive correlation with the following GAG degree (r=0.333), EGSS (r=0.431) and Itching scale(r=0.847) (Table 3).

Table (3): Correlation between CRP, Il-31, and demographic & clinical score among studied cases.

		CRP (mg/l)	IL-31(ng/l)
Age/years	rs	-0.142	-0.170
	P value	0.381	0.295
BMI (kg/m^2)	rs	**-0.371**	0.111
	P value	**0.018***	0.497
Onset of disease /years	rs	**-0.445**	0.017
	P value	**0.004***	0.919
GAG score	rs	0.291	0.246
	P value	0.068	0.126
GAG degree	rs	**0.356**	0.333
	P value	**0.024***	**0.036***
EGSS	rs	**0.343**	**0.431**
	P value	**0.03***	**0.005***
Itching scale	rs	0.170	**0.847**
	P value	0.296	**<0.001***

rs: Spearman correlation coefficient. *Statistically significant.

There was a statistically significantly higher median CRP among cases with severe and very severe GAG degrees than the group with moderate and the least is detected for the group with mild degrees (p-value 0.03). There was a statistically significantly higher median IL-31 among cases with severe and very severe GAG degree than the group with mild and the least is detected for the group with moderate degree (p-value 0.03) (Table 4).

Table (4): Relation between CRP & GAG and IL31 & GAG degree among studied cases

	GAG Degree			P-value
	Mild n=11	Moderate n=13	Severe & Very severe n=16	
CRP (mg/l) median (min-max)	0.2±0.02	0.7±0.1	3.65±0.91	0.03*
IL-31(ng/l) median (min-max)	1079±246.32	821±189.74	1555±322.91	0.03*

Table (5 and Fig. 3) show that the area under the ROC Curve of CRP &IL-31 for differentiating cases with acne vulgaris from the control group is fair with the best detected cut off points being 0.650 &911 respectively yielding a sensitivity of 65 & 67.5 and specificity of 52.5% &50%, respectively.

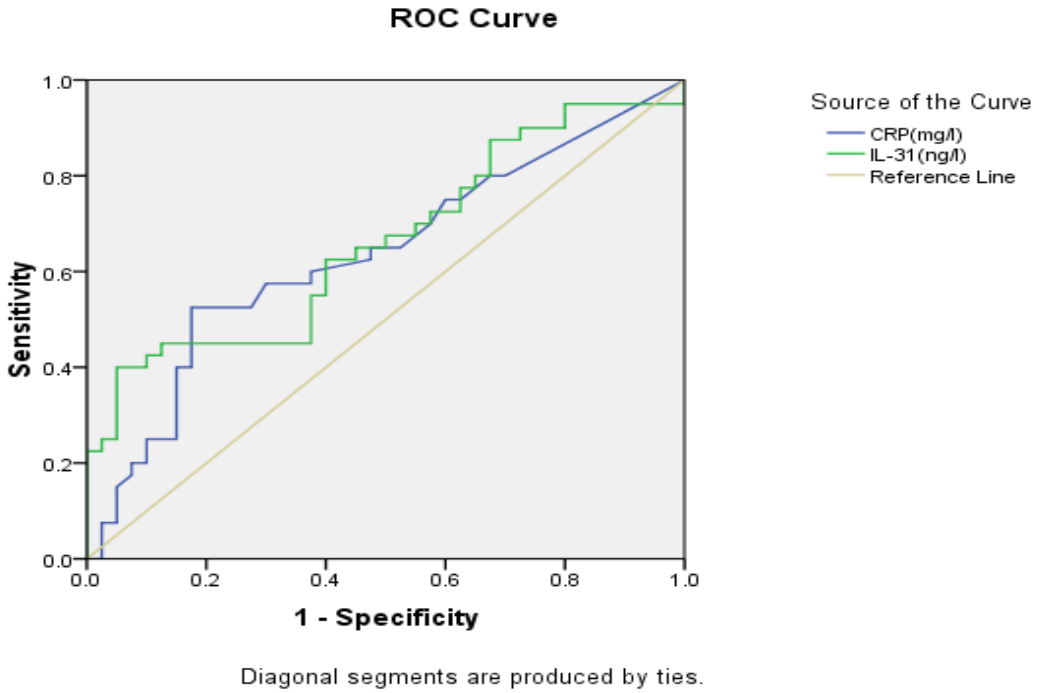

Figure (3): ROC curve of CRP & IL-31 in differentiating cases with acne.

Table (5): Validity of CRP & IL-31 in differentiating cases with acne

Test Result Variable(s)	Area	P-value	Asymptotic 95% Confidence Interval		Cut off point	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
CRP(mg/l)	0.636	0.036*	0.513	0.759	0.650	65.0	52.5
IL-31(ng/l)	0.661	0.013*	0.541	0.781	911.0	67.5	50.0

DISCUSSION

In the present study, there was no statistically significant difference between the studied groups as regards their age, sex, and body mass index with the mean age of the studied cases being 25 versus 23.3 among the control group. Seventy percent of cases were females versus 62.5% of the control group. The mean body mass index of the studied cases was 24.55 versus 24.43 among the control group.This mean age was in accordance with **Chatzikonstantinou *et al.* [8] and Mazzetti *et al.* [9]. Mazzetti *et al.* [9]** found that the mean age at diagnosis of acne vulgaris was 24.4 years. While, some studies had a dissimilar mean age of participants a lower mean age ranging from 14.7 to 16.7 years [10, 11].

Our result revealed that female was slightly predominant. The female: male ratio in our patient group was 70%: 30%. Similarly, **Sevimli Dikicier [12]** found of their patients 178 were females (71.2%) and 72 were males (28.8%). In contrast, **Kaushik *et al.* [13]** found that males were twice as affected by acne than females. These results may be due to differences in the characteristics of the sampled population or country studied **[14].** In our study, the mean body mass index of the studied cases was 24.55. Most studies have noted an increased prevalence of acne in overweight and obese individuals (typically defined as BMI $\geq$23kg/m^2 and BMI $\geq$25kg/m^2, respectively) relative to underweight individuals (BMI <18.5kg/m^2) or individuals of a normal weight (18.5kg/m$^2\leq$BMI<23kg/m^2) **[14].** Obese and overweight individuals tend to have higher glycemic loads and androgen levels, which may increase sebum secretion, promoting the formation of acne lesions **[15].**

In the current study, 55%of the studied cases have a positive family history of acne vulgaris. Studies have also demonstrated the impact of genetic factors on acne presentation **[14]. Dreno and Poli [16]** reported that a positive family history of acne in parents was associated with increased acne risk in their offspring. Similarly, positive family history of acne was reported by 59.8% in the **Kaminsky *et al.* [17]** study.

In our study, the GAG degree distribution among studied cases is 35% severe, 32.5% moderate, 27.5% mild and 5% very severe. This was inconsistent with the findings of **Kaushik *et al.* [13]**, who reported eighty-two percent of their study participants had mild acne while 16% had moderate acne according to the GAGS score and only 2.5% had severe acne.

In our study, the median age of disease onset among studied cases is 4 ranging from 2 to 16 years, the median GAG score is 23 ranging from 11 to 52 and the median itching scale is 2 ranging from 0 to 8. According to GAGS, the mean acne severity was 21.5 ± 7.4 in the **Eyüboglu *et al.* [18]** study. More than half (52.5%) of the patients had scores less than 5 in the itching scoring system and 47.5% had scores of 5 or more in the **Ramadan *et al.* [4]** study.

In the present study, there was a statistically significantly higher median IL-31 among studied cases than in the control group (1078.5 versus 912.5). Similarly, **Ramadan *et al.* [4]** found that IL-31 levels were significantly higher in both patients' tissue and serum when compared with controls (P=0.001). Interleukin-31 (IL-31) is a novel T-helper-lymphocyte-derived cytokine with an established role in inducing inflammation and pruritus **[19].**

To the best of our knowledge, there was only a single paper that previously discussed the role of IL-31 in the context of acne vulgaris.

Our result demonstrated that there was a statistically significantly higher median CRP among studied cases than in the control group (2.47 versus 0.55). In line with this, **Monib *et al.* [20]** found that the serum and salivary levels of CRP in the patients were significantly higher than the measured levels in the control subjects ($p < 0.001$). This result was also in agreement with the findings of **Mohammed *et al.* [21] and El-Taweel *et al.* [22].**C- reactive protein (CRP), one of the acute phase proteins, is the best indicator of systemic inflammation, as its level elevates rapidly in cases of inflammation, and its serum levels show no circadian changes across the day. IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α) which are implicated in the pathogenesis of acne are also major inducers of CRP production by the liver. Thus, CRP levels could be elevated in acne if the amount of inflammation is high enough **[23].** On the other hand, **Namazi *et al.* [5]** did not detect an elevated serum CRP level in their acne vulgaris patients. They concluded that acne vulgaris, even in its severe grades, is not one of the conditions which induce significant inflammation at the systemic level. **T**he nonsignificant difference between patients and controls in their study can be explained by the nature of the control group. They recruited the control patients from the blood donors who were not exactly matching the patients. They also suggested that the inflammatory reaction of acne might be a completely local reaction not affecting the systemic levels of the inflammatory markers.

In our study, there was a statistically significant negative correlation between CRP and the BMI & onset of disease (r=-0.371 & -0.445) and a statistically significant positive correlation with GAG degree & EGSS (r=0.356 & 0.343). This was following **Namazi *et al.* [5] and Monib *et al.* [20],** the serum CRP levels showed a significant positive correlation with GAGS scores.

In our study, IL-31(ng/l) showed a statistically significant positive correlation with the following GAG degree (r=0.333), EGSS (r=0.431) and Itching scale(r=0.847). In agreement with our result, **Ramadan *et al.*** [(4)] found a statistically significant correlation between mean tissue level of IL-31 and severity of acne supporting the role of IL-31 in the inflammation **but no** statistically significant correlation was found between itching score and levels of IL-31 in both tissue (P=0.127) and serum (P=0.127).

In the current study, a statistically significantly higher median CRP among cases with severe and very severe GAG degree than group with moderate and the least is detected for the group with mild degree (3.65, 0.7 &0.2, respectively). Similarly, **Namazi *et al.*** [(5)] found that the mean CRP levels in the severe acne group were higher than that of the moderate acne group.

In the instant study, a statistically significantly higher median IL-31 among cases with severe and very severe GAG degrees than the group with a mild degree, and the least is detected for the group with moderate degree (1555, 1079 &821, respectively). This can be explained by the chemotactic properties of IL-31, which can effectively induce chemokines, IL-6, IL-16, and IL-32, which are important in inflammation. It was also reported that IL-31 has a role in the induction of matrix metalloproteinases [(24)].

The present study demonstrated that; the detection of CRP & IL-31 levels could be used as a reliable indicator in differentiating studied cases with the best-detected cut-off point being 0.650 & 911 respectively yielding a sensitivity of 65 & 67.5 and specificity of 52.5% & 50%, respectively.

CONCLUSIONS

This work suggested a pathogenic role of IL-31 in acne patients being related to the severity and inflammation, opening gates for a better understanding of the pathogenesis of common dermatological disease and thus better treatment results.

Serum levels of CRP are elevated in acne vulgaris. CRP correlated with the disease severity.

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