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Correlation of IL-8 and C - Reactive Protein Serum Levels with the Severity of Inflammatory Acne Vulgaris: A Comparative Study

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Abstract:

Background: *Inflammatory Acne vulgaris (IAV) involves high Interleukin-8 (IL-8) serum level. To date, no study was conducted to highlight the correlation of IL-8 and C-reactive protein (CRP) with IAV.*

Objective: *this study was conducted to investigate the correlation of IL-8 and CRP serum levels with IAV regarding the severity of the disease.*

Materials and Methods: *Sera of 68 patients with IAV and 70 gender and age matched healthy volunteers were enrolled. IL-8 and CRP were measured in patients' sera with enzyme linked immune sorbent assay (ELISA). The levels were correlated with the severity of the disease using Global Evaluation Acne (GEA) scale.*

Results: *IL-8 and CPR serum levels in patients with IAV were significantly higher than controls ($p<0.05$). There were high statistically significant differences of IL-8 and CPR levels with severity of disease ($p<0.05$).*

Conclusion: *In IAV, IL-8 and CRP are proportionally correlating to the severity of inflammatory acne vulgaris. Blocking IL-8 and CPR production may hold promise therapy in limiting the deleterious effects of IL-8 and CPR mediated inflammatory response.*

Keywords: *ELISA, Inflammatory Acne vulgaris, IL-8, C-reactive protein, Global Evaluation Acne scale.*

1. INTRODUCTION

Acne vulgaris is a common skin disease of the pilosebaceous unit. It is characterized by non-inflammatory lesions as seborrhea, comedones (blackheads and whiteheads); inflammatory lesions as papules, pustules and nodules; and possibly scarring [1].

The pathogenesis of inflammatory acne vulgaris (IAV) is multifactorial and complex; including hormonal, microbiological, and immunological mechanisms [2]. The interaction between *Propionibacterium* acnes (*P*. acnes) and infiltrated monocytes and lymphocytes also play an important role in the pathogenesis of IAV [3, 4]. Interleukin-8 (IL-8) is a CXC-type chemokine that binds to the cellular seven transmembrane domain G protein-coupled receptors known as CXCR1 and CXCR2 [5, 6]. IL-8 is a potent proinflammatory chemotactic factor that predominantly exerts its chemotactic effects on neutrophils [6-8].

IL-8 is produced by activating blood monocytes and tissue macrophages such as Kupffer cells (specialized tissue macrophage). It acts primarily as a co-stimulant for Th1 cells and induces the production of IFN-γ, IL-2 and granulocyte macrophage colony stimulating factor (GM-CSF). High IL-8 level is associated with *P*. acnes and *P*. acnes elected Kupffer cells [4]. Also macrophages enhance liver to synthesize C reactive protein (CRP) which is considered as a marker of inflammation [9-10] and is associated with acne [11-12]. CRP induces IL-8 mRNA secretion from human blood monocytes [13].

As IL-8 and CRP association with IAV are approved, this study was conducted to investigate whether these associations were correlated proportionally with the severity of the disease or not.

2. OBJECTIVE

This study was conducted to investigate the correlation between serum high level of IL-8 and CRP with the severity of the disease.

3. SUBJECTS AND METHODS

3.1. Subjects

This descriptive comparative study was carried out in Dermatology outpatient clinic, Suez Canal University Hospital for a period of 6 months in accordance with the guidelines of the Helsinki Declaration, and was performed after obtaining the informed consent from all parents of the children and patients.

Out of 157 patients with IAV; 73 excluded because either, having pregnancy, lactating women, receiving medical treatment, having other skin diseases, having other systemic diseases or those who refused to participate. By simple randomization of 84 patients with IAV eligible to participate in the study, only 68 patients with IAV enrolled. Age and gender matched 70 healthy volunteers enrolled as a control group.

3.2. Methods

All of the studied patients were subjected to full history-taking, general and systemic examination with special emphasis on the presence or absence of IAV and staging the severity of the disease according to Global Evaluation Acne (GEA) scale [14]. Serum levels of IL-8 a CRP were measured in all subjects by ELISA (a polystyrene Microtiter plate).

3.2.1. IL-8 assay

The microtiter plate provided in this kit has been precoated with an antibody specific to IL-8 Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-8 and Avidin conjugated to Horse-radish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB (3, 3'5, 5' tetramethyl-benzidine) substrate solution was added to each well. Only those wells that contain IL-8, biotin-conjugated antibody and enzyme-conjugated Avidin would exhibit a change in color. The enzyme substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of IL-8 in the samples was then determined by comparing the O.D. of the samples to the standard curve [15].

3.2.2. CRP assay

Wells of the microtitre plate were coated with polyclonal antibodies against CRP. Firstly in incubation, the CRP in the samples was bound to the coated polyclonal rabbit antibodies. By washing step, all unbound substances removed. In a second incubation step, we added Peroxidase-labeled CRP antibody. After another washing step, all unbound substances were removed; the solid phase was incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stopping solution was then added. The color converted to yellow. Yellow color's intensity was directly proportional to the concentration of CRP in the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration was generated. CRP, present in the patient samples, was determined directly from this calibration curve [16]

3.3. Statistical Analysis

Statistical analysis was carried out using computer program SPSS version 16 (Statistical Package for the Social Science; SPSS; Inc., Chicago, IL, USA). A probability value (p-value) <0.05 was considered statistically significant.

4. RESULTS

In 138 subjects, there were (61.8 %) females and (38.2 %) males with a mean age of 20.6 ± 3.7 years. Moderate IAV appeared in 38 patients (55.9 %), 12 patients with mild and other 12 patients with severe form were in equal percent with (17.6 %). Almost clear cases and very severe cases were (6 %)

and (3%) respectively. IL-8 serum levels in patients with IAV were significantly higher than control (308.4 ± 420.4 pg/ml versus 22.3±12.1 pg/ml, respectively) [Table (1)]. CRP serum levels in patients with IAV were significantly higher than control (6.9 ± 4.9 mg/l versus 1.3 ± 1.5 mg/l, respectively) [Table (1)]. The relation between severity of IAV and age and gender of patient were non-significant (p = 0.65 and p =0.30, respectively). Table (2) showed a highly statistically significant differences between IL-8 level and CRP with severity of disease (p= 0.001 and p=0.01, respectively). Results showed moderate positive correlation between severity of IAV with IL-8 level and CRP (r = 0.63 and r = 0.71 respectively), which was statistically significant (p < 0.05). Also, a weak positive correlation between IL-8 and CRP with duration of disease (r = 0.29 and r = 0.30 respectively), which was statistically significant (p < 0.05). In contrast, there was a weak positive correlation between severity of IAV and age and gender regarding IL-8 and CRP level. Simultaneous multiple regression was conducted to investigate the best predictor of IL-8 level and CRP. The inter-correlations of the entered variable among all study subjects were shown in [table (3, 4)]. The combinations of variables were to predict IL-8 level and CRP included age, gender, duration of disease and severity of disease, which were (F (4.64) = 13.82, p < 0.001 and F (3.67) = 12.83, p < 0.001, respectively).

The beta coefficients were presented in [table (5, 6)]. Note that the duration and severity of the disease significantly predict IL-8 level and CRP when all four variables are included. The adjusted R2 value of IL-8 was 0.43 this means that 43 % of the variance of IL-8 was explained by the model. That for CRP was 0.42 and means that 42% of the variance of CRP was explained by the model.

Table1. *Assessment levels of IL-8 (pg/ml) and CRP (mg/l) among patients with IAV and controls.*

	IL-8 (pg/ml)		CRP (mg/l)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Patients with IAV	308.4 ± 420.4	12 - 950	6.9 ± 4.9	0 - 56
Controls	22.3 ±12.1	11 - 58	1.3 ± 1.5	0 - 6
Statistical test ^{MW}	428.5		342.5	
p-value*	0.04		0.02	

MW = Mann Whitney test was used

**significant at p-value < 0.05.*

Table2. *Relation between (IL-8 and CRP) levels and disease severity.*

Disease severity		**IL-8 level**	**CRP level**
		Mean ± SD	**Mean ± SD**
Controls (no acne)		22.3 ± 12.1	2.3 ± 1.5
patients with IAV	**Almost clear**	12.5 ± 0.7	0.5 ± 1.4
	Mild	29.5 ± 16.4	2.2 ± 3.1
	Moderate	205.9 ± 368.7	4.3 ± 4.9
	Severe	906.7 ± 40.8	5.9 ± 1.5
	Very severe	930 ± 23.7	7.1 ± 5.5
Statistical test value^{KW}		14.73	9.60
p-value*		0.001	0.01

KW= Kruskal Wallis test.

**statistically significant at p-value < 0.05.*

Table3. *Intercorrelations between IL-8 and the predictor variables.*

	Age	**Gender**	**Severity of disease**	**Duration of disease**
IL-8	- 0.18	0.06	0.63**	0.29**
Age		-0.08	- 0.14	0.21*
Gender			- 0.01	- 0.09
Severity of disease				0.74**
Duration of disease				

**p-value < 0.05*

***p-value < 0.01*

Table4. *Intercorrelations between CRP and the predictor variables.*

	Age	Gender	Severity of disease	Duration of disease
CRP	- 0.14	0.08	0.71**	0.30**
Age		-0.07	- 0.13	0.25*
Gender			- 0.02	- 0.08
Severity of disease				0.64**
Duration of disease				

**p-value < 0.05*

***p-value < 0.01*

Table5. *Multiple regression analysis summary for age, gender, severity of disease and duration of disease predicting IL-8 level.*

	B	St error of B	Beta (β)	p-value*
Age	3.05	7.71	0.04	0.69
Gender	23.38	63.49	0.03	0.71
Severity of disease	191.29	31.03	0.94	< 0.001
Duration of disease	- 45.57	17.16	- 0.41	0.01
Constant	- 116.19	202.38		0.57

Note: *R^2= 0.43, F (4.64) = 13.82, p < 0.001.*

** Significant at p-value < 0.05*

Table6. *Multiple regression analysis summary for age, gender, severity of disease and duration of disease predicting CRP level.*

	B	St error of B	Beta (β)	p-value*
Age	2.05	6.51	0.03	0.78
Gender	32.28	56.59	0.02	0.65
Severity of disease	292.39	32.03	0.93	< 0.001
Duration of disease	- 46.47	15.15	- 0.42	0.01
Constant	- 126.18	301.37		0.58

Note: *R^2=0.42, F (3.67) = 12.83, p < 0.001.*

** Significant at p-value < 0.05*

5. DISCUSSION

Sebum overproduction due to sebaceous gland hyperplasia occurs firstly in IAV [17]. Also, the hair follicle hyperkeratinizes and prevents normal shedding of the follicular keratinocytes. Subsequent obstruction of the follicle forms an unapparent microcomedo. Thus, lipids and cellular debris accumulate within the blocked follicle [18]. Colonization of *P*. acne is encouraged by this microenvironment and provokes the immune response after production of numerous inflammatory mediators. Follicular rupture, leakage of lipids, bacteria and fatty acids into the dermis enhances inflammation.

Vowels et al., [19] and Chen et al [20] demonstrated that *P*. acnes induced IL-8 production, which is one of the main proinflammatory mediators produced by monocytes [21]. IL-8 gene is the one most highly induced by all bacterial stimulants [22]. So, IL-8 is the key proinflammatory mediator in innate immunity against bacteria by inducing a profound neutrophil chemoattraction. Lim et al also showed that CRP highly produces cytokines such as TNF-α, IL-1, IL-6 and IL-8 from monocytes [23]. IL-8 plays a key role in the initiation of vascular inflammation [6-8]. CRP not only can induce IL-8 mRNA and potentiates secretion elevation from human blood monocytes, but also affects the whole circulation to increase the IL-8 level [13]. Data from this study pointed out the increased level of IL-8 and CRP in the serum of patients with IAV versus healthy subjects indicating the pivotal role of inflammation in IAV. Results of this study agreed with previous studies which showing that IL-8 was released by a different of cell types including monocytes, macrophages, T lymphocytes, fibroblasts, endothelial cells and keratinocytes in response to inflammatory stimuli [3, 22, 24].

IL-8 attracts and degranulates neutrophils [25, 26]. Subsequently; potential key regulators of cell signaling released as cathepsin G, serine proteases, proteinase and leucocyte elastase at sites of inflammation. This followed by activating different IL-8 receptors [27]. This point was supported in the present study by the presence of a highly significant moderate positive correlation (r = 0.36, p = 0.003) between the severity of acne and IL-8 level was found. This agreed with **Zhong-yong** and his colleagues **[**28]

Kim et al. [29] reported that toll-like receptor 2 (TLR-2) has been implicated in the pathogenesis of IAV. TLR-2 was activated by *P*. acnes. When bound, TLR-2 activated a transcription factor that up regulates production and the release of proinflammatory cytokines like IL-12 and IL-8 from monocytes. TLR-2 was expressed on infiltrating inflammatory cells around the pilosebaceous follicle. Its expression increased as the acne lesion ages and became more inflamed. This agreed with result of our study, as there was a significant weak positive correlation between duration of disease and IL-8 level. In contrast to this study; Abd El All et al [30] reported that the duration of the disease was not affected by IL-8 expression. In 2005 Nagy et al explained activation of TLR-2 and hypothesized that *P*. acnes induced IL-8 secretion could have a key role in initiation of inflammatory events in acne by attracting neutrophils to the site of active lesions [31]. Also, CRP in the studied groups was found statistically significant elevation and also there was statistical significance correlation between CRP elevation and severity of the disease. On the other hands, these results disagreed with Vergou T et al [16]. Thus, both high elevation of IL-8 and CRP had pivotal role in sever form of IAV.

6. CONCLUSION

IL-8 and CRP are proportionally correlates to the severity of inflammatory acne vulgaris. Its presence in IAV may contribute to the host defenses against *P. acnes* as well as to tissue damage through its various actions of the involved immune cells and inflammatory mediators. Targeted therapy to block IL-8 and CPR production may hold promise in limiting the deleterious effects of IL-8 and CPR mediated inflammatory response.

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