Assessment of IL-12 Serum Level in Patients with Inflammatory Acne Vulgaris and its Correlation with its Severity

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Abstract

Background: Inflammatory Acne vulgaris (IAV) involves colonization of Propionibacterium acnes (P. acnes). P. acnes up-regulates of neutrophils, monocytes and lymphocytes with several cytokines being released locally.

Objectives: IL-12 is associated with IAV; we investigated whether this association was correlated with the severity of the disease.

Material and Methods: Sera of 27 patients with IAV and 27 gender and age matched healthy volunteers were enrolled. IL-12 and C-reactive protein (CRP) were measured in patients' sera with enzyme linked immune sorbent assay (ELISA). The level was correlated with the severity of the disease using Global Evaluation Acne (GEA) scale.

Results: IL-12 serum levels in patients with IAV were significantly higher than controls (p<0.05). But, CRP serum levels in patients with IAV were non-significantly different from control. There was non significant difference as regard relation between IL-12 level and severity of disease (p> 0.05), also, non statistical significant difference as regard relation between CRP level and severity of disease (p> 0.05).

Conclusion: In IAV, IL-12 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. Blocking IL-12 production may hold promise therapy in limiting the deleterious effects of IL-12 mediated inflammatory response.

Introduction

Acne is a multifactorial disease of as yet incompletely elucidated etiology and pathogenesis [1]. The combination of keratin, sebum and microorganism particularly P. acnes leads to release of proinflammatory mediators and accumulation of T-helper lymphocytes, neutrophils and foreign body giant cells. This in turn causes the formation of inflammatory papules, pustules and nodulocystic lesions [2].

Acne vulgaris is a common skin disease of the pilosebaceous unit. It is characterized by seborrhea, comedones (blackheads and white-
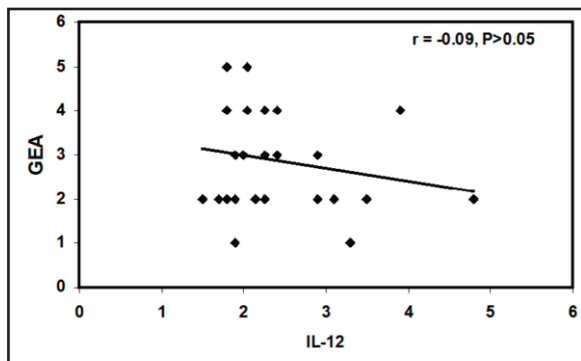
Figure 1. Negative correlation between levels of IL-12 and severity of the disease
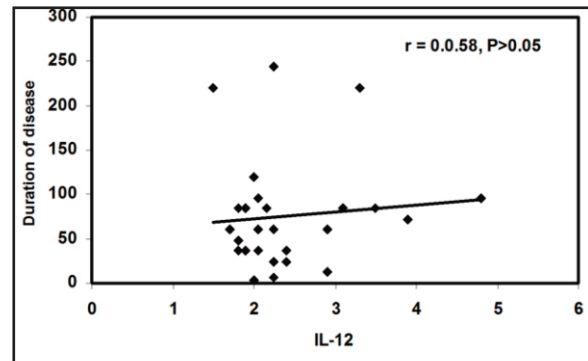

Figure 2. Showed positive correlation between levels of IL-12 and duration of the disease
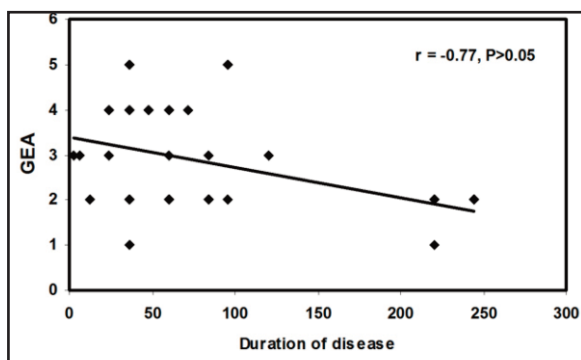

Figure 3. Correlation between duration and severity of the disease

heads) which are the non-inflammatory lesions; papules (pinheads), pustules (pimples) and nodules which are the inflammatory lesions, and possibly scarring [3].

The pathogenesis of inflammatory acne vulgaris (IAV) are multifactorial and complex; including hormonal, microbiological, and immunological mechanisms [4]. The interaction between P. acnes and infiltrated monocytes and lymphocytes may also play an important role in the pathogenesis of IAV. [5, 6]. IL-12 acts as a stimulatory factor on Th 1 cells. Th 1 cells can be derived from the exposure of neonatal CD 4+ cells to IL-12. Re-stimulation of differentiated Th 1 populations with a single dose of IL-12 resulted in the emergence of IFN-γ producing Th 1 cells and IFN-γ/ IL-4-producing Th 0 cells. However, Th 1 cells retained their phenotype even following two rounds of IL-12 treatment [7].

P. acnes induce IL-12 release from TLR2 positive monocytes and from peripheral blood mononuclear cells [8].

As IL-12 is associated with IAV, we investigated whether this association was correlated with the severity of the disease.

Materials and Methods

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.

By interviewing with 157 patients with IAV; 73 excluded because either, pregnant and lactating women, receiving medical treatment, having other skin diseases, having other systemic diseases or those who refused to participate. By simple randomization of 85 patients with IAV eligible to participate in the study, only 27 patients with IAV enrolled. Other 27 gender and age matched healthy volunteers enrolled as a control group.

Methods: All of the studied patients were subjected to the following: 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 [9, 10]. Serum levels of IL-12 a C-reactive protein were measured in all subjects by ELISA (a polystyrene Microtiter plate).

IL-12 assay: The microtiter plate provided in this kit has been precoated with an antibody specific to IL-12 standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-12 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-12, 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-12

Table 1. Assessment Levels of IL-12 (pg/ml) and CRP (mg/l) Patients with IAV and Controls

	IL-12 (pg/ml)		CRP (mg/l)	
	Mean ± SD	Range	Mean ± SD	Range
Patients with IAV	2.4 ± 0.75	1 – 3.7	2.9 ± 3.9	0 - 20
Controls	1.8 ± 0.4	0.2 – 2.1	2.3 ± 1.5	0 - 6
Statistical test MW	3.234		542.5	
p-value*	0.002		0.52	

MW = Mann Whitney test was used; *significant at p-value < 0.05

Table 2. Relation Between (IL-12 and CRP) Levels and Disease Severity

Disease severity		IL-12 level	CRP level
		Mean ± SD	Mean ± SD
Controls (no acne)		1.8 ± 0.4	2.3 ± 1.5
Patients with IAV	Almost clear	1.8 ± 0.7	0.5 ± 1.4
	Mild	2.3 ± 1.5	4.2 ± 3.1
	Moderate	3.3 ± 0.22	3.3 ± 4.9
	Severe	5.2 ± 0.35	1.9 ± 1.5
	Very severe	6.1	1
Statistical test value (KW)		3.735	7.60
p-value*		0.001	0.13

KW= Kruskal Wallis test; *statistically significant at p-value < 0.05

Table 3. Intercorrelations Between IL-12 and the Predictor Variables

	Age	Gender	Severity of disease	Duration of disease
IL-12	- 0.16	0.05	0.09**	0.58**
Age		-0.09	- 0.17	0.22*
Gender			- 0.14	- 0.08
Severity of disease				0.77**
Duration of disease				

*p-value < 0.05; **p-value < 0.01

in the samples was then determined by comparing the O.D. of the samples to the standard curve [**11**].

C-reactive protein assay: Wells of the microtitre plate were coated with polyclonal antibodies against C - reactive protein. Firstly in an 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.

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.

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-12 serum levels in patients with IAV were significantly higher than control (2.4 ± 0.75 pg/ml versus 1.8 ± 0.4 pg/ml, respectively). On the other hand; C - reactive protein serum levels in patients with IAV were non-significantly different from control (2.9 ± 3.9 mg/l versus 2.3 ± 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). A negative statistically significant difference as regard relation between IL-12 level and severity of disease (p < 0.05) and also no statistical significant difference as regard relation between CRP level and severity of disease (p= 0.13) were shown (**Table 2** and **Figure 1**). Results showed moderate positive correlation between severity of IAV and IL-12 level (r = 0.09), which was statistically significant (p < 0.05). Also, a weak positive correlation between IL-12 and duration of disease (r = 0.58), which was statistically significant (p < 0.05) (**Figure 2**). In contrast, There was a weak positive correlation between severity of IAV and age and gender (r = 0.17 and r = 0.14, respectively), which was statistically significant (p = 0.14 and p = 0.27, respectively). Simultaneous multiple regression was conducted to investigate the best predictor of IL-12 level. The inter-correlations of the entered variable among all study subjects were shown (**Table 3** and **Figure 3**).

Discussion

This study was conducted to assess the correlation of IL-12 to the severity of IAV for more understanding of its role (as a part of cell mediated immunity and pro inflammatory mediators) in pathogenesis of acne. This study enrolled 27 patients with acne and 27 healthy persons of the same gender and age [22 females (81.5%) and 5 males (18.5%)], ranged from12 up to 42 years to assess serum IL-12 in all age groups of IAV.

The result of this study pointed to significant increase in the mean serum levels of IL-12 in the IAV group versus control group, which reflect the role of IL-12 in inflammatory response. This agreed with *Kim* et al, *Ramos* et al and *Sugisaki* et al who detected significant increase in levels of IL-12 and other cytokines including IL-2,-6, -8 and INF-γ [**4, 12, 13**]. IL-12 induces Th1 immune response (Th1 direction), also stimulates the production of IFN-γ from NK cells and inhibits IL-4 (Th2 direction) as reported by [**14**]. By immunohistochemistry, [**4**]. detected that TLR 2 was markedly increased in cells of acne lesions associated with significant increase in IL-12 and IL-8 levels, that proved immune cells such as TLR 2 expressing macrophages (perifollicular and peribulbar) tend to surround pilosebaceous follicles in acne lesions, subsequent TLR 2 triggering due to P. acnes resulted in IL-12, IL-8, IL-6 and IL18 cytokine production [**15**]. By acting of Propionibacterium acnes on TLR-2, could stimulate the secretion of cytokines, such as IL-8 and -12 in macrophages giving rise to inflammation. Certain P. acnes species may induce an immunological reaction by stimulating the production of sebocyte and keratinocyte antimicrobial peptides, which play an important role in the innate immunity of the follicle [**16**].

This study showed non significant correlation between the gender of patients and severity of the disease, and this agreed with *Ghodsi* et al [**17**]. On the other hand, in 2009 *Adityan* and *Thappa* [**18**], observed in their study that male patients had more severe acne vulgaris than female patients. The males also had a significant higher prevalence of severe acne in results of *Hanisah* et al [**19**]. Also in our study, the correlation between age of patients and severity of the disease was non significant and that agreed with *Palmer* [**20**].

There was also statistically negative non significant correlation between serum IL-12 and severity of acne, non significant positive correlation was detected in our study between serum IL-12 and duration of the disease, the lack of correlation between IL-12 and acne severity suggested that this cytokine may be essential for development of all inflammatory

papule independent of the degree of severity. Similarly, failure to correlate serum levels of IL-I2 with patient's age and disease duration implied that IL-12 may possibly be one of the early and essential contributors to the development and maintenance of inflammation of IAV, along with other cytokines described, also *Ramos* et al, [**12**] detected non significant correlation between local IL-12 and duration of the disease, IL-18 [pro inflammatory cytokine (Th1 mediator) as IL-12] also, there was a non significant correlation between its level and (duration &severity) of the disease *Zhong-Yong* et al, [**21**], there was no association between serum TNF α genotypes and severity of acne detected by *Baz* et al, [**22**]. and *Anwar* et al, [**23**].

Although IL-8 acts as IL-12 in acne pathogenesis as pro inflammatory cytokines but significant association had been identified between increased dermal IL-8 expression and the severity of the disease, but not with the duration of the disease [**24**].

Also, IL-12 was detected in serum of dermatological auto immune patients other than acne patients, *Rossi* et al, [**25**] detected a significant increase in the serum IL-12 level in patients of progressive alopecia areata than of normal control ones, psoriasis [**26**], cutaneous lupus erythematosus [**27**], multiple sclerosis [**28**], Behçet's disease [**29**], so IL-12 shares in pathogenesis of multiple autoimmune skin diseases. Serum IL-12 is increased in associated with systemic autoimmune diseases such as rheumatoid arthritis [**30**], Crohn's disease [**31**] and others.

Conclusion

IL-12 is a potent pro-inflammatory cytokine and but not correlated to its severity. 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-12 production may hold promise in limiting the deleterious effects of IL-12 mediated inflammatory response.

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