

Serum Cytokine Levels in Patients with Chronic Atopic Dermatitis: a Useful Clinical Marker for Disease Activity

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Abstract

Objective: To measure the serum level of IL4, IL10 and IFN-gamma of Atopic patients before and after treatment with tacrolimus ointment or topical steroid by using enzyme linked immunosorbant assay (ELISA) to evaluate the disease activity.

Methods: IL4, IL10 and IFN-gamma concentrations in the serum were measured by enzyme-linked immunosorbent assay in fifty atopic dermatitis patients were giving two types of treatment and 43/50 were re-analyzed after one month of treatment with tacrolimus or topical and systemic steroids. The severity of atopic dermatitis was graded on the criteria of Hanifin and Rajika. The disease activity was assessed by each patient on a visual analogue scale (VAS).

Results: Higher serum IFN- γ , IL-10 and IL-4 concentrations were found in AD patients sera at the time of diagnosis and correlated with disease activity before and after treatment. Serum IFN- γ , IL-10 and IL-4 concentrations also correlated with VAS scores for itching, skin condition; overall skin symptoms and total VAS score. Serum IFN- γ concentration could be a good indicator to define the degree of the general activity in chronic AD patients, also IFN- γ can play a role in the immuno-pathogenesis of AD, and furthermore may be used in the diagnosis and follow up of the disease in addition to other parameters.

Conclusion: The sera of patients group revealed a statistically significant increase in the concentration of IFN-gamma, IL-4 and IL-10 before treatment when compared with that of the same patients after treatment with topical tacrolimus-steroid and also there were no statistically significant differences between two treatments.

Keywords: Atopic dermatitis, interleukins, interferon gamma, tacrolimus

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Introduction

Atopic dermatitis is a chronic inflammatory skin disease with a complex pathogenesis. It is clinically well-defined and represents one manifestation of the atopic state, along with asthma and/or allergic rhinitis [1].

Atopic dermatitis is a chronically relapsing eczematous skin disease resulting from complex interactions between genetic

and environmental factors. A large number of immunological and non-immunological abnormalities have been reported in AD patients whether in the skin or serum or even urine [2].

Acute AD is associated with the production of T helper type 2 (Th2) cytokines, notably IL-4 and IL-13 [3]. Which mediate immunoglobulin isotype switching to IgE synthesis, and up-regulate expression

of adhesion molecules on endothelial cells. In contrast, IL-5, is involved in eosinophil development and survival, and predominates in chronic AD [4].

The maintenance of chronic AD also involves the production of the Th1-like cytokines IL-12 which produced by langerhans cells, eosinophils and keratinocytes appears to be a predominant mediator for the induction of IFN- γ in T-cells after homing to skin [5].

Tacrolimus (FK504) ointment is widely used in the treatment of patients with AD [6]. The drug exerts its action by down-regulating antigen specific T-cell activities and associated pro-inflammatory cytokine production [7].

Methods

Fifty atopic dermatitis patients (30 female and 20 male) were giving two types of treatment and 43/50 were re-analyzed after one month of treatment with tacrolimus or topical and systemic steroids. These patients who attended to private clinic during the period extended from May 2008 to September 2008. Patients were selected randomly and the mean age of patients 26 ± 11 years and range from 6-45 years, most frequent age group was in second decade group (55%). Most of the patients (74%) were presented with high disease activity. Patients were diagnosed as an atopic dermatitis according to the diagnostic criteria of Hanifin and Rajika [8]. Specimens of blood were collected from them by following the guide-lines of WHO (1995).

To investigate whether the patients were in allergic status and apart from suggestive clinical data, blood samples from all subjects were tested for total serum IgE titer and blood eosinophil count before and after treatment. Enzyme linked Immunosorbent Assay (ELISA) was used for the measurement of the total IgE in sera of the studied groups, Anti-Human IgE peroxidase

conjugate IgG antibody was used for this purpose, The procedure of ELISA is making according to the Hunter et al 1986. The results were expressed in IU/ml and by cut of value were expressed as positive or negative.

Blood Samples Processing

A sterile needle inserted into the vein and the required volume of blood (3-5ml) was withdrawn, and was transferred to serum tube (free of anticoagulant) and let to coagulate for serum preparation. After completing coagulation the serum separated using centrifuge 3000 RPM for 5 minutes.

Serum was collected and kept in a labeled appendroff tubes at -20 C° to be used for serological studies. Blood sample collected in EDTA tube which was used for studying the following hematological tests.

ELISA quantitative measurement of human IL-4, IL-10 and IFN- γ in serum:

Kits:

The following diagnostic kits were used during the study:

- 1- Anti-human IgE peroxidase conjugate; Antibody developed in Goat IgG fraction of Antiserum; product No. A9667. (SIGMA)
- 2- IL-4 ELISA kit (BIOSOURCE IL-4 EASIA KIT) ; Catalogue Number KAC1281.
3. IL-10 ELISA kit (BIOSOURCE IL-10 EASIA KIT) ; Catalogue No. KAC1321.
- 4-IFN-gamma ELISA kit (BIOSOURCE IFN-g EASIA KIT); Catalogue No. KAC1231

Sadwich ELISA for measurement of human IL-4, IL-10 and IFN- γ in serum:

We developed an efficient ELISA method for measurement of human IL-4, IL-10 and IFN- γ in serum which has already been introduced to the market by medical and biological laboratories co., Ltd, BioSource Europe S.A. Nivelles Belgium. Standards and controls were performed in duplicate for

each sample, and the mean values were reported.

Statistical Analysis

Statistical analysis was performed with the SPSS 15.01 statistical package for social sciences and also Excell 2003. Data analysis was done by using paired sample t-test for tables with pretreatment and post treatment data means, independent sample t- test if we have two different groups. P-value of ≤ 0.05 was used as the level of significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation (SD) and standard error (SE).

Cut-off value was measured by calculation the upper limit of the 99% confidence interval, which calculated by the calculation of the mean of the (OD-values) of standard readings (M) and the stander deviation (SD) and the stander error (SE).

$$\text{Cut-off value} = M + 2.57(\text{SD} \times \text{SE}).$$

Pearson correlation was done to explore possible association between markers involved in the study [9].

Results

Eosinophil count:

Figure (1): shows results of estimation and comparison of eosinophil count in which a highly significant difference is recorded among AD group at time of diagnosis when compared with those after treatment and was elevated in most AD patient correlating roughly with the disease severity.

Also there was significant difference between pre-treatment group and post-treatment group to evaluate the disease activity.

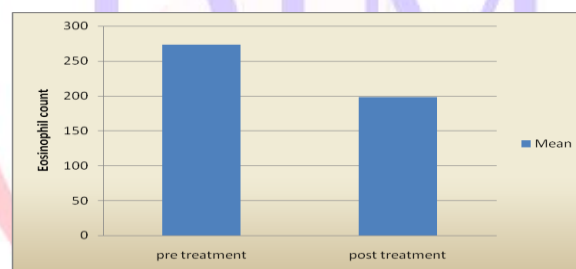


Figure (1): Mean of eosinophil count pre & post-treatment.

The total serum IgE:

The determination of total IgE in the serum was performed by using sandwich ELISA for all subjects and the results in Figure (2), show that AD patient's serum

contain significant higher level at time of diagnosis when compared with that of post-treatment group. Furthermore, there was highly significant difference between pre-treatment group and post-treatment group.

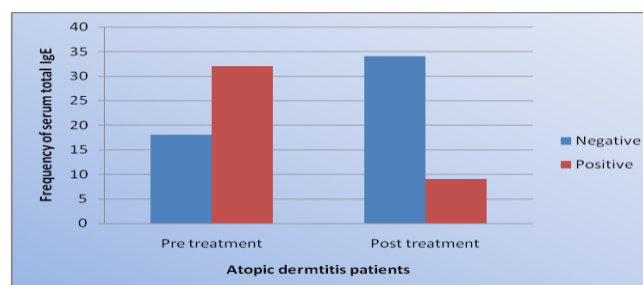


Figure (2): Total serum IgE frequency among pre-treatment and post-treatment AD patients groups.

Serum IL-10: Serum of our patients contain higher levels of IL-10 at time of diagnosis than did post treatment serum group, and analysis of T-test revealed that there was

statistical significant difference between pre-treatment and post-treatment groups ($p \leq 0.05$) as shown in table (2).

Table (1): Mean serum IL-10 concentration (pre and post-treatment group).

ELISA		Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
Serum IL-10	pre treatment	185.860	264.043	40.266	0.002*
	post treatment	50.628	40.353	6.154	

*statistical significant difference ($p \leq 0.05$).

Serum IL-4: IL-4 concentration was especially increased in AD patients at time of diagnosis as compared with post-treatment group. Analysis of T-test revealed that there

was statistical significant difference between pre-treatment and post-treatment groups ($p \leq 0.05$). See table (3).

Table (2): Mean Serum IL-4 level.

ELISA		Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
Serum IL-4	pre treatment	83.907	17.597	2.684	0.006*
	post treatment	75.558	23.324	3.557	

/*statistical significant difference ($p \leq 0.05$).

Serum interferon –gamma (IFN- γ): Serum of most patients with chronic AD and those with active or moderate disease actively contain higher levels of IFN- γ at time of diagnosis than did post treatment serum

group and analysis of T-test revealed that there was statistical significant difference between pre-treatment and post-treatment groups ($p \leq 0.05$), see table (3).

Table (3): Mean serum IFN- γ concentration in two treatment group.

ELISA		Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
Serum IFN- γ	pre treatment	6.797	7.543	1.150	0.004*
	post treatment	3.818	5.096	0.777	

///*statistical significant difference ($p \leq 0.05$).

Effect of steroid-tacrolimus on serum IL-4, IL-10 and IFN- γ

One month after treatment with steroid-tacrolimus therapy, the skin lesions regressed. See table (6) showed that there

was no significant difference could be found between the effects of both drugs on serum and urine IL-4, IL-10 and IFN- γ before and after treatment. Also see figure 3.

Table (5): Effect of steroid-tacrolimus on serum IL-4, IL-10 and IFN- γ .

pre treatment	drug	N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
Serum IFN-gamma	Steroid	23	8.220	8.120	3.631	0.359
	Tacrolimus	5	12.980	7.342	3.284	
Serum IL-4	Steroid	5	89.800	8.497	3.800	0.278
	Tacrolimus	5	83.400	8.905	3.982	
Serum IL-10	Steroid	5	448.000	558.397	249.723	0.241
	Tacrolimus	5	122.800	131.506	58.811	

Relationships between serum IL-4, IL-10 and IFN- γ and total VAS score:

As table (7) and figure (3) show the serum IL-10,IL-4 and IFN- γ and total VAS score :

significantly correlated with each symptoms, which was the sum of three individual skin aspects (itching, skin dryness, skin condition) P value ($P < 0.05$).

Table (6): relationships between serum IL-4, IL-10 and IFN- γ and total VAS score:

		VAS (itching skin condition)
Serum IFN- γ	pretreatment	$P < 0.05$ (*)
	Post treatment	$P < 0.05$ (*)
Serum IL-4	pretreatment	$P < 0.05$ (*)
	Post treatment	$P < 0.05$ (*)
Serum IL-10	pretreatment	$P < 0.05$ (*)
	Post treatment	$P < 0.05$ (*)

Significant correlation, P value ($P < 0.05$).



Leg eczema (pre-treatment)



Leg eczema (post-treatment)

Figure (3): clinical pictures before and after treatment with tacrolimus ointment.

Discussion

Eosinophil count: In our study, blood eosinophilia is presented in most patients with AD correlating roughly with the disease severity.

Total IgE: The total IgE – serum level was found to be higher positive in AD patients at time of diagnosis when compared with post-treatment group.

Serum cytokine levels (such as IFN- γ , IL-4 and IL-10) were shown to be increased in patients with chronic AD at time of diagnosis. We tested whether serum concentrations of these cytokines are useful inflammatory markers for assessing AD severity in children and adult. To investigate this, we assessed the severity of AD clinically by VAS score, and also correlated these cytokines concentrations before and after treatment.

In this study, the results showed that serum IFN- γ concentration in chronic AD patients was higher at time of diagnosis (mean value=6.797 \pm 7.543) than post-treatment group (mean value=3.818 \pm 5.096) with statistical significant difference among them ($p\leq 0.05$). It's seems to be specifically increased among chronic AD patients. Such finding comes in agreement with studies of Antunez, 2006 who recorded the higher level of IFN- γ concentration in chronic AD sera, at time of diagnosis.

Serum IL-10 levels were also higher before treatment (mean value = 185.860 \pm 264.043) compared with serum levels after treatment (mean value =50.628 \pm 40.353) with statistical significant difference ($p\leq 0.05$), while the serum levels of IL-4 showed weaker correlation with activity of the disease at time of diagnosis (mean value=83.9 \pm 17.59) and post-treatment group (mean value=75.5 \pm 23.324).

So these results showed that the serum levels of IFN- γ can be a sensitive parameter that importantly correlated not only with

clinical severity of AD, but also strongly associated with other clinical and laboratory disease activity markers like eosinophile count and total IgE.

So Serum cytokines may be a useful inflammatory markers for assessing severity of AD.

From previous ongoing discussion about serum IFN-g and its association with clinical and laboratory parameters, it's worthy enough to suggest that serum IFN-g as a good therapeutic target for AD. This furthermore argued by huge body of board studies that used IFN-g blocking agent. [8]

All previous facts put serum IFN-g on the map as a valid biomarker for chronic AD patients and the treatment with IFN-g blocking agent should be used for patients suffering from active disease. Since, there are indications that those therapeutic agents should be used in the early "window of opportunity" to help patients with AD achieve long term, and sustained improvement.

In our study, we used the new immune-modulator topical drug (tacrolimus ointment) at the first time in Iraq in treatment of atopic dermatitis and compared with the old traditional topical steroids therapy for AD. We investigated the effects of tacrolimus ointment on chronic AD lesions and compared with effects of topical steroids (Betamethasone valerate) clinically and immunologically.

Immunologically; our study showed that tacrolimus and topical steroid inhibited Th2 cytokines (IL4 and IL-10) in serum and Th1 cytokines (IFN- γ) in serum. Suppressive effects of tacrolimus on cytokine production was equal to that of betamethasone valerate.

Conclusions

Higher serum IFN- γ , IL-10 and IL-4 concentrations were found in AD patients sera at the time of diagnosis and correlated with disease activity before and after

treatment. Serum IFN- γ concentration could be a good indicator to define the degree of the general activity in chronic AD patients, also IFN- γ can play a role in the immunopathogenesis of AD, and furthermore may be used in the diagnosis and follow up of the disease in addition to other parameters.

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