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## Research Communication

# The Relationship Between Serum Levels of Total IgE, IL-18, IL-12, IFN- $\gamma$ and Disease Severity in Children With Atopic Dermatitis

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Studies about the role of cytokines on the immunopathogenesis of atopic dermatitis (AD) are generally based on in vitro observations and this role has not been completely clarified yet. Serum levels of total IgE, IL-18, IL-12, IFN- $\gamma$  and the relationship between these parameters and disease severity, determined using the SCORAD index, in a group of atopic patients were investigated in this study. Serum levels of total IgE were measured by the nephelometric method and serum levels of IL-18, IL-12/p40 and IFN- $\gamma$  were measured by ELISA method. Serum levels of total IgE and IL-18 were found significantly higher in study group than in controls ( $P < .001$ ). There was no statistically significant difference between patients and controls in respect of serum levels of IL-12/p40 ( $P = .227$ ). A statistically significant relationship between SCORAD values and serum levels of total IgE ( $P < .001$ ), IL-18 ( $P < .001$ ), and IL-12/p40 ( $P < .001$ ) was determined. These results show that serum levels of IL-18 can be a sensitive parameter that importantly correlates with clinical severity of AD, 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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## INTRODUCTION

Atopic dermatitis (AD) is a frequently encountered, chronic, severely itchy, eczematous, and inflammatory skin disease that can seriously affect health quality. Encounter frequency of this disease is increasing gradually and the latest data about the immunopathogenesis of the disease have led to the development of new effective treatment models [1–3].

Many patients with AD (80–90%) have high serum levels of IgE [4]. When IgE binds to the surface antigens of mast cells, mast cells degranulate and the released mediators cause dermatitis to be formed. It is determined in recent times that IgE causes IL-1, IL-3, IL-4, IL-5, IL-6, GM-CSF, and TNF- $\alpha$  to be synthesized and released by binding to basophils and mast cells. These cytokines play an important role in the late phase of allergic response [5]. Local cytokine profile is a critical determinant of AD skin lesions [6]. TNF- $\alpha$ , one of these cytokines, inhibits the growth of Th1 cells and this can cause immune order to spoil. Most of the lymphocytes in the skin with AD secrete Th2 cytokines and IL-10, IL-4, IL-13 [7].

Expression of GM-CSF mRNA and IL-12/p40 subunits is increased in chronic atopic dermatitis skin lesions compared to normal skin [8]. These cytokine profiles require a Th2 dominant environment in the continuity of AD lesions. Besides, IL-4 and IL-13 induce IgE production by Ig heavy chain regulation and reduce lymphocyte and eosinophil production by VCAM-1 increase [9]. These findings correlate with clinical signs of AD [10].

IL-18 is a recently defined member of IL-1 cytokine family and was known as IFN- $\gamma$  stimulating factor before. Later studies put forward that it has wide range effects other than lymphocyte activation. So, it is understood that IL-18 is an important regulator in the production of both innate and acquired immune responses [11, 12]. IL-18 directly regulates the effects of T and B cells, NK cells, macrophages, dendritic cells (DC), and chondrocytes [13]. Progression of type-1 lymphocyte and NK cell responses occurs basically by synergic participation of IL-12. But this synergism is probably specific because type-2 response can be formed by IL-18 without IL-12 existence [11, 12, 14]. In addition to this, wide range

effects of IL-18 on functions of macrophages and dendritic cells occur directly independent from IFN- $\gamma$  or indirectly by the mediation of cytokines produced by T cells and NK cells. IL-18 can also induce production of IgE and Th2 cytokines as synergistically with IL-2 [15, 16]. We can see reverse effects of IL-18 like being able to crossregulate Th1/Th2 derivation also in IL-12 [17–19].

Studies about the role of cytokines on formation of atopic diseases are rather new and most of them are based on in vitro observations. It is not completely clear yet how cytokines regulate diseases in vivo and studies about this subject are rather limited. Furthermore, the severity of AD is based on clinical observations and there is no objective laboratory parameter to assess the severity. Serum levels of total IgE, IL-18, IL-12, IFN- $\gamma$  and the relationship between these parameters and the disease severity in a group of child patients with AD were investigated in this study.

## MATERIAL AND METHODS

### Patients

Twenty consecutive child patients with AD who applied to dermatology clinic of KSU (5–12 year old; 11 males, 9 females) were included in this study as study group and 19 volunteers who have no dermatologic or systemic disease and were in accordance with study group in respect of age (5–11 year old; 10 males, 9 females) were included in this study as control group. AD patients were determined according to Hanifin and Rajka criteria [20]. Disease history was taken from patients or their parents. Patients who received ultraviolet therapy or immunotherapy before, who received local or systemic therapy in last 3 weeks, or who have any other systemic, allergic, parasitic, or dermatologic disease were excluded from this study.

### Assessment of disease severity

The severity of AD was assessed by the same dermatologist using the Scoring Atopic Dermatitis (SCORAD) index system [21].

### Measuring serum levels of total IgE and cytokines

Blood samples taken from AD patients (study group) and healthy individuals (control group) were kept at room temperature for 30 minutes till they coagulate. Then they were centrifuged at 2000 rpm for 10 minutes and serums were obtained. These serums were preserved at  $-70^{\circ}\text{C}$  and dissolved just before the analysis. Total IgE levels were measured by the nephelometric method (Dade Behring Marburg GmbH, Marburg, Germany). IL-18, IL-12 (by IL-12 p40 kit), and IFN- $\gamma$  levels were measured by ELISA method (BioSource Europe SA, 8 B-1400 Nivelles, Belgium). The minimal detection levels of cytokines using these methods were 12.5 pg/mL for IL-18 (inter- and intra-assay reproducibilities were %8.54 and %4.93); < 4 pg/mL for IFN- $\gamma$  (inter- and intra-assay precisions were %6.0 and %5.2) and < 2 pg/mL for IL-12 p40

subunit (inter- and intra-assay precisions were %3.9 and %3.9).

### Statistical analysis

All variables were expressed as mean values  $\pm$  SD, median, and range. Undetected cytokine levels were set at the minimum detectable concentrations. Nonparametric statistical methods were applied to continuous variables. Mann-Whitney U test and Spearman's nonparametric correlation test were used for statistical analysis. *P* values < .05 were considered statistically significant. Analyses were performed by using SPSS software, version 11.0 for Windows (SPSS Inc, Chicago, IL).

## RESULTS

The SCORAD values were found between 3 and 35 (mean  $\pm$  SD:  $12 \pm 7$ ) in the patients. Serum total IgE levels were statistically significantly higher in study group (176–847 IU/mL) than in control group (18–81 IU/mL) (*P* < .001). Serum levels of IL-18 were determined in all patients in the study group but could be determined in only 19 of healthy individuals in the control group. Serum levels of IL-18 were determined statistically and were found significantly higher in patients (23–81 pg/mL) than controls (< 12.5–73 pg/mL) (*P* < .001). Serum levels of IL-12/p40 were determined in 11 of 20 AD patients in the study group and in 8 of healthy individuals in the control group. There was not a statistically significant difference between patients (< 2–93 pg/mL) and controls (< 2–52 pg/mL) in respect of serum levels of IL-12/p40 (*P* = .227). Serum levels of IFN- $\gamma$  could not be determined in any of the patients in the study group or healthy individuals. Results are shown in Table 1.

A statistically significant relationship between serum levels of total IgE (*r* = 0.99; *P* < .001), IL-18 (*r* = 0.99; *P* < .001), IL-12/p40 (*r* = 0.84; *P* < .001), and SCORADs in children with AD was determined. Also, the relationship between serum levels of total IgE and serum levels of IL-18 (*r* = 0.99; *P* < .001) and IL-12/p40 (*r* = 0.85; *P* < .001) in patients with AD was determined to be statistically significant. The relationship between the disease severity and serum levels of total IgE, IL-12/p40, IL-18 is presented in Table 2.

## DISCUSSION

Serum IgE level is often increased in allergic diseases. But a normal serum IgE level does not mean that atopy does not exist. On the other hand, very high serum IgE level can be accepted as one of the important data showing allergy existence if there is no parasitic infestation [22]. Increased IgE level, basophile and mast cell degranulations, and released mediators are important for AD but these are secondary features with cAMP function disorders. Although IgE has a role on formation of allergic rhinitis and some forms of allergic asthma, there are few publications in the literature that IgE has a major role on the pathogenesis of AD. It is stated in

TABLE 1: Serum levels of total IgE, IL-18, and IL-12/p40 in control group and in patient group.

Groups	Total IgE ( IU/mL)	IL-18 ( pg/mL)	IL-12 ( pg/mL)
Patient group ( $n = 20$ )	275, (176–847)	68, (23–81)	31, (< 2–93)
Median, (minimum-maximum)			
Control group ( $n = 19$ )	32, (18–81)	< 12.5, (< 12.5–73)	< 2, (< 2–52)
Median, (minimum-maximum)			
<i>P</i> value	$P < .001$	$P < .001$	$P = .227$

TABLE 2: Relationship between SCORAD values and serum levels of total IgE, IL-18, IL-12/p40.

SCORAD, total IgE, and cytokines	Statistical values	SCORAD	Total IgE	IL-18
Total IgE	<i>R</i>	.99	—	—
	<i>P</i>	< .001	—	—
IL-18	<i>R</i>	.99	.99	—
	<i>P</i>	< .001	< .001	—
IL-12/p40	<i>R</i>	.84	.85	.85
	<i>P</i>	< .001	< .001	< .001

the literature that serum IgE levels correlate with AD severity [1, 23, 24]. Laske and Niggemann performed a retrospective study on 345 child patients with AD and determined that SCORAD values were statistically significantly related with serum IgE levels [25]. It was determined in our study that serum total IgE levels were below 200 IU/mL in only 4 of 20 patients with AD and they were statistically significantly higher in study group than in control group. Also a statistically significant relationship was determined between SCORAD values and serum total IgE levels.

IL-18 acts synergistically with IL-12 to stimulate the development of Th1 cells in in vivo conditions. It has been shown that IL-18 also increases Th2 cytokine production, eosinophily, and allergic sensitivity in ragweed-antigen stimulated models [26]. It induces IL-13 that is a Th2 cytokine and makes this induction synergistically with IL-12 in nonexistence of IFN- $\gamma$  [15]. It is determined that IL-13 is the major determinant of serum IgE levels in patients with AD [6, 27]. It is shown in a study performed by Hoshino et al about stimulation of Th2 response by IL-18 mediation that IL-18 induces IgE production independently from IL-4 in in vivo conditions [16]. Shida et al found that serum IL-12/p40 levels were higher in AD patients than in controls and there was a relationship between serum IL-12/p40 levels [28]. These researchers separated IL-18 functionally into type-1 (active) and type-2 (inactive) and while they determined type-1 IL-18 in all AD patients, they determined type-2 IL-18 only in 3 AD patients with low serum IgE levels. Because of this, they put forward that type-2 IL-18 may be reciprocally related with serum IgE levels in the patients. They found serum IFN- $\gamma$  levels below determination limits in all patients. It was determined in a study performed by Yoshizawa et al that there was no statistically significant difference between AD patients and controls in respect of serum IL-12 levels but serum IL-18 levels were higher in AD patients than in controls [29]. It is also determined in this study that serum IL-18 levels correlate with clinical activity of AD. It is determined

in a study performed on 19 child patients with AD that SCORAD scores were statistically significantly related with serum IL-18 levels and serum IFN- $\gamma$  levels could not be determined in patients with AD and in controls [30].

We found in our study that serum IL-18 levels were statistically significantly higher in AD patients than in control group. On the other hand, we could not determine a statistically significant difference between AD patients and healthy controls in respect of serum IL-12/p40 levels. We found that SCORAD values were statistically significantly related with serum levels of IL-18 and IL-12/p40. The relationship between serum total IgE levels and serum levels of IL-18 and IL-12/p40 was also determined to be statistically significant in AD patients.

Serum levels of IFN- $\gamma$  were determined below determination limit in both study group and control group. Serum levels of IFN- $\gamma$  and IL-12 increase in chronic phase lesions of AD but they are measured at the same level with unaffected skin in acute phase lesions of AD [31]. These two parameters might be found in normal limits in this study because of most of the patients in our study being in the acute phase of AD. High serum IL-18 levels make us think that IL-18 is a sensitive parameter that correlates with clinical severity of AD and may play a role on immunopathogenesis of AD.

These results show that serum levels of IL-18 and IgE may be used as important markers in the assessment of disease severity and follow-up of child patients with AD. We believe that these kinds of studies will give clues on determining diagnosis, treatment, and protection strategies about AD in the future. As a result, the role of cytokines and the relationship between cytokines in the immunopathogenesis of AD are rather complex and still not clearly clarified. Especially, when in vitro and in vivo functional variation of IL-18, the complexity of bioactivities triggered by IL-18 and interactions between IL-18 and other cytokines are considered, further investigations are required to understand this complex process.

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