

## Evaluation of serum interleukin- 35 level in children with persistent asthma

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### Abstract

**Background:** IL-35 is a novel cytokine suppressing the immune response through the expansion of regulatory T cells and suppression of Th17 cell development. Few studies have been done on the effect of this cytokine in a different disease. Asthma is a complex disease that many inflammatory cells and cytokines play a role in.

**Objective:** We decide to determine the difference between serum level of IL-35 in childhood asthma & healthy children.

**Method:** We obtained serum samples from 44 asthmatic children between 2 and 15 years as a case group and from healthy children as a control group. IL-35 serum concentration was determined by ELISA method in both groups.

**Results:** Mean serum level is 30.9 pg/ml in the case group and 30.2 pg/ml in the control group. There is no significant difference between serum level of IL-35 in asthmatic & healthy children.

**Conclusions:** Our data reveal no relation between childhood asthma and serum level of IL35. So, further study will be needed to clarify effects of this cytokine in human allergic diseases.

**Keywords:** IL-35, asthma, immune regulation, allergic disease, Th 17

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### Introduction

The most common respiratory disease is asthma that many cells and inflammatory markers have a role in this disease.<sup>1</sup> Over times this disease has significant morbidity and shows an increasing prevalence. According to Global Initiative for Asthma (GINA) as many as 300 million people worldwide suffer from asthma.<sup>2</sup> This chronic disease has complex pathogenesis and criteria for the diagnosis of asthma are variable according to the age. In preschool-aged children, symptom patterns are important for diagnosis,<sup>3</sup> but in older children the diagnosis can make on symptoms, physical exam, and spirometry.<sup>4</sup> Airway inflammation in asthmatic patients involve polarization of the T lymphocyte response to the Th2 cells so Th2 plays a major role in pathogenesis of the inflammatory process in asthma,

although recent studies indicate that pathological mechanisms of asthma involve more than just a dichotomous Th1/Th2 inflammation.<sup>5</sup>

By releasing cytokines Th2 cells recruit inflammatory cells such as mast cell and eosinophils into the airways. Significant infiltration of neutrophils has been observed in severe asthma and Th17 cells are thought to be the central mediator of the recruitment of neutrophils. Also, another subtype of CD4 T cells, the regulatory T cells have an anti-inflammatory activity by blocking TH2 responses.<sup>6,7</sup> These cells have a major role in allergic disease and produce cytokines that have an immunomodulatory function such as IL-10 and TGF- $\beta$ .<sup>8</sup> Interleukin -35 (IL-35) is the newest member of the IL-12

family; this family primarily consisted of IL -12, IL-23 and IL-27. The source of IL-35 is FOXP3 Treg.<sup>9,10</sup> The action of this cytokine is suppression of immune system by inducing the proliferation of Treg cells and inhibiting the differentiation of TH17 cells.<sup>11</sup> Also in a recent study, authors show that IL-35 produced by plasma cells can provide a novel opportunity for immune intervention and can be used in therapeutic procedures. They demonstrated that IL-35 that produced by plasma cells had suppressing roll during salmonella infection, on the other hand this cytokine can protect mouse during EAE.<sup>12</sup>

In a mouse model of an airway inflammation induced by dust mite allergen, IL-35 can limit airway inflammation and IgE production suggesting that IL-35 had potential of suppressing allergic lung inflammation, which was mediated by Th2 cells as decreasing production of IgE and IgG4.<sup>13</sup>

Furthermore, another investigation proved that IL-35 has regulatory activity of Tregs by showing that IL-35 produced by T reg can inhibit generating T cells proliferation.<sup>14</sup> In humans, IL-35 has been suggested to involve with disease pathogenesis in several diseases such as chronic hepatitis B, coronary artery disease, portal hypertension, autoimmune diabetes, and pancreatic disease.<sup>15-20</sup>

However, the majority of the studies were conducted in a murine model and there were only a few published studies in human.<sup>21</sup> Although decreased concentration of IL-35 in plasma of patients with asthma and COPD was indicated previously,<sup>22</sup> study focuses on the plasma concentration of IL-35 and its possible roles in allergic disease was lacking.

Particularly, asthma that starts in adulthood differs from childhood onset of asthma, the typical difference is that adult asthma is rarely originated from allergic condition, moreover adult asthma is more severe and patients usually experience earlier decline of lung function.<sup>23</sup> Due to these differences in pathogenesis of both diseases, we focus only in childhood asthma in this study to evaluate the possible role of IL-35 in asthma pathogenesis. Therefore, in this study, we compared the serum concentration of IL-35 in children with asthma and healthy subjects to investigate any relationship between IL-35 and asthma.

## Methods

### Demography

Forty-four children (aged 8±3 years, ranged: 2-15) who referred to Imam Reza Clinic (Shiraz University of Medical Science, Shiraz, Iran) newly diagnosed with asthma from October 2012 to October 2013 were enrolled. The study protocol was approved by Shiraz University Ethics Committee (code: 93608). All the patients were visited by two different allergists, separately. They all fulfilled the criteria of persistent asthma based on the asthma guidelines.<sup>4</sup> Asthma severity was evaluated on the basis of the Expert Panel Report 3 (EPR3) guidelines.<sup>4</sup> Patients with upper respiratory tract infections and those with other serious illness except for asthma and allergic rhinitis were excluded from the study. None of the asthmatic patients recruited had undergone an inhaled corticosteroid (ICS) therapy before having their blood tested and Pulmonary function was evaluated by spirometry (Cosmed, Rome, Italy) in patients older than 6 years. Forced vital capacity (FVC),

forced expiratory volume in 1 second (FEV1), ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) and peak expiratory flow (PEF) were measured, and values less than 80% of the predicted value were considered abnormal. Information about demographic characteristics, passive smoking behavior, family history of atopy and other concomitant allergic diseases such as allergic rhinitis and atopic dermatitis was collected by questionnaire.

Forty –four sex and age matched unrelated healthy individuals with no personal or family history of asthma and other atopic diseases were selected as a control group of the same ethnicity and from the same geographic region (Fars province, southwestern Iran).

### Sample collection and ELISA:

Blood samples were obtained after getting informed consent. 5 cc peripheral blood from patients were obtained and serum samples were isolated immediately and stored at -80°C until required for an analysis by commercial ELISA Kit (USCN Life Science, US) according to manufacturer instruction. All samples were assayed two times in one laboratory. On the other hand, all the sera were evaluated for IgE concentration by ELISA method.

### Statistical analysis

Data analysis was carried out by using SPSS software (version 16, US). We used a t-test to compare the average of case and control groups. To analyze the normal distribution of interleukin in two groups, Kolmogorov-Smirnov test were applied. But due to abnormal distribution of data in two groups,

We used also Kolmogorov-Smirnov test. Coefficients for plasma IL-35 concentrations and other possible correlated factor (allergic rhinitis, atopic dermatitis, history of smoking in the family, parental asthma, and serum IgE level) were determined by using two tests: t-test and Mann-Whitney test. Differences were considered to be significant when  $p < 0.05$ .

## Results

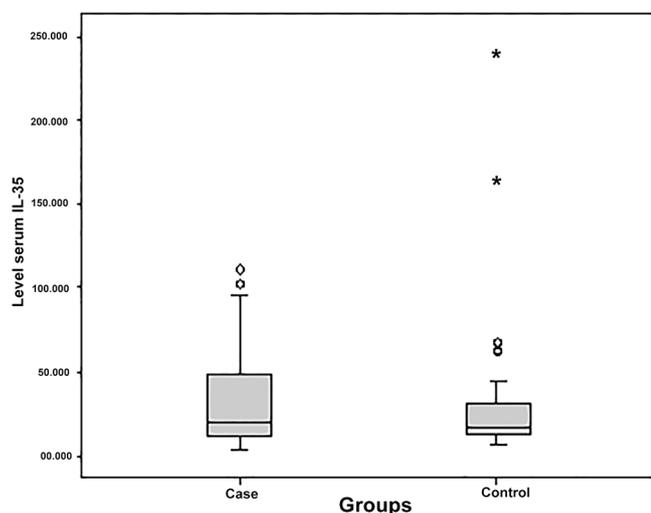
To identify whether IL-35 level in asthmatic children, 44 children the newly diagnosis of asthma who were not receiving any medication were selected according to inclusion criteria. All the patients were between 2 and 16 years with mean age 8.8±3.5. The cause of higher percentage of boys (52%) in the study was consistent with the epidemiology of asthma in this age group. There were no significant differences in age and gender between the asthmatic patients and healthy control group. The characteristics of the patients are summarized in **table 1**.

In asthmatic children, the mean concentration of plasma IL-35 was 30.9 pg/ml; the highest concentration was 110.7 pg/ml and lowest 3.8pg/ml. In the control group, the mean value of plasma IL-35 concentration was 30.2 that the highest concentration was 239.7 pg/ml and lowest 6.1pg/ml. Further analysis showed that there is no significant difference in values of plasma IL-35 in asthmatic group and control subject although control group had a higher concentration of serum IL-35 as it can be seen in **Figure 1**.

Asthmatic patients had were divided into 3 groups (mild, moderate, and severe) according to EPR3 criteria, and based

**Table 1. Demographic and clinical characteristics of patients with asthma and control group are showed.** Control group was adjusted to normal subjects. Control group data had not data such spirometry or data about asthma severity because they were normal subjects.

	Patients	Control group
Sex	N= 44	N= 44
Females	21(48%)	21(48%)
Males	23(52%)	23(52%)
Age		
<10 years	20(46%)	20(46%)
>10 years	24(54%)	24(54%)
Severity		
Mild	35(86%)	
Moderate	5(9%)	
Severe	4(11%)	
Spirometry indices	N=36	
FEV<80%	30(68%)	
FVC<80%	35(79%)	
FEV1/FVC<80%	22(50%)	
PEF<80%	40(91%)	
Smoking status	N=44	N=44
Passive smoker	20(46%)	15(34%)
Parental asthma	N=44	N=44
Asthmatic parents	26(60%)	17(38%)
Non asthmatic	18(40%)	27(62%)

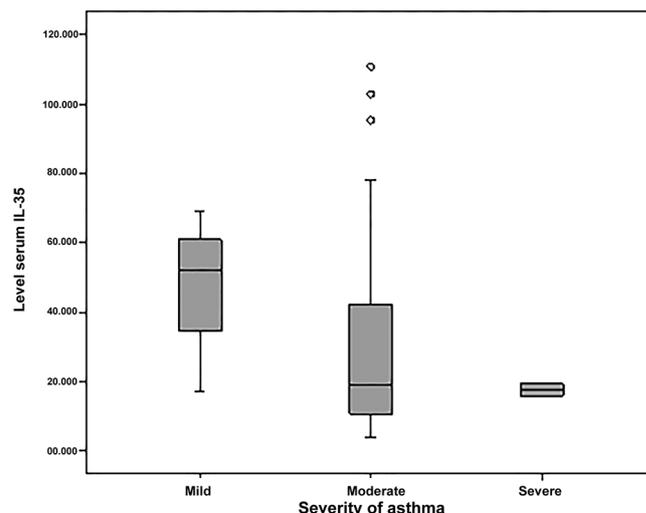


**Figure 1. Comparison between mean concentration of sera IL-35 in asthma and control group<sup>1</sup>**

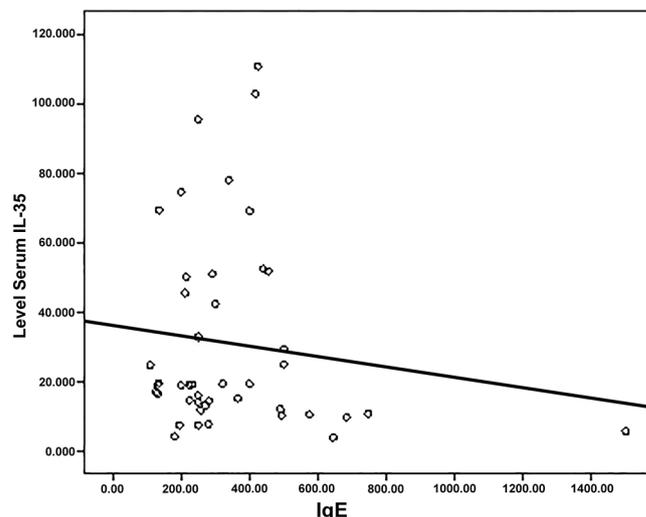
on the asthma condition were independently evaluated for relation with IL-35 serum level. It can be vividly seen in **Figure 2** that there is no significant relation between any groups and concentration of IL-35.

Evaluating the relation between IL-35 and serum IgE level had shown that there is no significant relationship between this interleukin and IgE, it can be seen in **Figure 3**.

Apart from this, no significant correlation was found between plasma IL-35 and other demographic characteristics like gender, age, and smoking history of their family (**Table 1**).



**Figure 2. Relation between asthma severity and mean concentration of IL-35**



**Figure 3. Relation between mean concentration of IgE and IL 35 in sera of asthmatic patients (P: 0.41 , r: 0.12)**

Moreover, no significant correlation was found between IL-35 level and allergic rhinitis or atopic dermatitis. In patients with dermatitis atopic the mean concentration of IL-35 was  $32.2 \pm 3$  and patients without a history of it was  $17.2 \pm 11.4$ . The outcome of analyzing the relation of allergic rhinitis with a concentration of this cytokine was  $30.6 \pm 29$  in a patient with no history of allergic rhinitis and was exactly the same for patients with a history of allergic rhinitis ( $31 \pm 29$ ).

### Discussion

IL-35 is a novel inhibitory cytokine which produced by Treg cells.<sup>14</sup> Monocytes, smooth muscle cells, and vascular endothelial cells are also potentiated to express IL-35 during inflammation.<sup>24</sup> IL-35 can mediate immunosuppressive functions and works as an inflammatory inhibitor in some autoimmune diseases, such as colitis (T-cell dependent) and arthritis (collagen-induced), but its effect in asthma and

COPD remain to investigate.<sup>25</sup> In an animal model study, it was observed that the intraperitoneal injection of IL-35 during allergen sensitization stage can be highly efficient. In another animal model study which evaluated NOD receptors during allergic asthma observed that granulocyte markers which involved in allergic asthma dramatically and positively correlated with plasma IL-35 concentration in these patients.<sup>26,27</sup>

In an investigation in 2015, it was revealed that a subset of CD4<sup>+</sup> classes, CD4<sup>+</sup>CD25<sup>-</sup> effector T cells has a key role in occurrence and persistence of airway inflammation in patients with allergic asthma. The IL-35 production can suppress IL-4-producing CD4<sup>+</sup>CD25<sup>-</sup> effector T cells and may associate with a decrease of IL-4. Rising IL-4 and also another cytokine like IL-5 cause Th2 cells induce eosinophil recruitment, mucus secretion, goblet cell hyperplasia and airway hyperresponsiveness.<sup>28</sup>

Furthermore, it has been suggested that asthmatic patients have decreased Treg cells while they increased Th<sub>2</sub> cells, which indicates that decreased IL-35 may be induced by the decline of Treg cells in the patients with asthma.<sup>10</sup> Some studies found that there was an obviously negative correlation between serum IL-35 and IL-4 levels. In addition, significantly positive correlation was also found between serum IL-35 and IFN- $\gamma$  levels.<sup>29</sup>

Our data showed that the plasma concentrations of IL-35 were detectable in all groups, and there is a slight decrease of IL-35 in allergic asthma patients in comparison with normal subjects; however, we could not observe a significant difference between asthmatic patients and healthy control group in IL-35 serum level. An investigation in 2014 also came to the same conclusion, it revealed that no significant relationship was found with IL-35 while desensitizing allergic mouse to dust mite to mouse condition. In their study, IL-35 level was not induced by immunotherapy procedure and IL-35 level remained unchanged.<sup>30</sup>

Some investigations analyzed the associations of IL-35 with IgE, eosinophil count, IL-4 and IFN- $\gamma$  and proved association among them,<sup>25</sup> but our study showed no significant correlation between IgE level and serum IL-35 level. On the other hand, according to our data, there is no apparent correlation between this cytokine and history of another allergic disease such as allergic rhinitis or atopic dermatitis. All these data may support its role in asthma or allergic condition but also may reveal that IL-35 does not play a key role in allergy. Although it should be considered that our patient only had a history of dermatitis and rhinitis, so they might have fluctuation in IL-35 in that period and was modulated during the time.

On the other hand, genetic and or lots of other factors that may affect IL-35 like none- Treg related factors which can elevate IL35 in healthy controls should be considered.

Our data indicated little difference in patients and healthy control group but the difference is not that much to become statistically significant, we should emphasize on that all of normal subjects and patients were controlled for general health and other issues like autoimmune diseases or viral infections. In comparing data to study by Chen C. et al 2014 we got different outcomes, the paradox in results is that our patients were children between 2-15 years old and have lower age than patients of that investigation that have mean age of 33 years old.

It is possible that the difference in pathogenesis of asthma in children and adult cause the variation of level of IL35.<sup>22</sup>

Another reason for our different result may be due to the difference in genetic background of patients, not only IL-35 polymorphism, but also all other related cytokine polymorphism may play a role in various data all over the world.<sup>31,32</sup> The other reason the difference may originate from is the difference in evaluating system and sensitivity of the methods or kits.

In our study showed that there is no correlation between asthma severity (mild, moderate, severe) and IL-35 level, it might due to lack of patients with severe asthma, as it can be clearly seen that most of the enrolled patients had moderate asthma. If more patients were enrolled to evaluate IL-35 level, it may give more power to reach the conclusion. Further researches on asthma in adults, severe asthma, or allergic asthma in all generation were required to better clarify this cytokine role in immune system.

## Conclusion

According to data, our study cannot strongly support a relation between children asthma and serum level of IL-35. However, more researches are necessary to clearly determine the role of IL-35 in asthma and evaluate the possible new strategies of therapies based on IL-35.

## Conflict of interest

There is none to be declared.

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