

## Comparison of Interleukin-33 Serum Levels in Asthmatic Patients with a Control Group and Relation with the Severity of the Disease

### Abstract

**Background:** The relation between interleukin-33 (IL-33) and asthma is not precisely known yet. The present study set to compare the serum level of IL-33 in patients with asthma and controls and study the relation with the severity of disease. **Methods:** The serum level of IL-33 and total IgE in 89 asthmatic patients and 57 controls were analyzed. The association of levels of IL-33 with the severity of disease, levels of total IgE, measures of spirometry (forced expiratory volume in 1 s [FEV1]), age, sex, presence or absence of other allergic diseases, and the disease duration was evaluated. **Results:** Higher levels of IL-33 and total IgE were detected in asthmatic patients compared with controls ( $P = 0.0001$  and  $P = 0.008$ , respectively). In the asthmatic group, a significant direct association of IL-33 with age ( $P = 0.02$ ,  $R = 0.23$ ) and with total IgE level ( $P = 0.003$ ,  $R = 0.31$ ) were observed, but there was no relationship between other variables. Comparison of mean level of IL-33 in different asthma groups concerning the disease severity showed the statistically significant difference between them and a significant increased serum level of total IgE was observed in more severe disease. The results showed a significant negative correlation between FEV1 and total IgE ( $P = 0.028$ ,  $R = -0.23$ ) and IL-33 level ( $P = 0.0001$ ,  $R = -0.83$ ). **Conclusions:** IL-33 is suggested as a new inflammatory marker of severe and refractory asthma. Therefore, it may be a unique therapeutic target in these patients.

**Keywords:** Asthma, interleukin-33, severity

### Introduction

Asthma is the lungs' chronic inflammatory disease characterized by variable airflow closure. Airway inflammation and injury are critical indicators of asthma pathogenesis.<sup>[1]</sup> In this regard, T-helper type 2 (TH2) lymphocyte-mediated immune responses and their cytokines are crucial in the disease pathogenesis. Interleukin (IL)-33 was used to be described as an IL-1 type cytokine associated with TH2 inflammation. However, since then, further evidence suggested the essential role of IL-33 in asthma and allergies as an initiator of the so-called responses.<sup>[2,3]</sup>

IL-33 expression increases in the epithelial cells and bronchoalveolar lavage fluid of bronchial asthma patients, as compared with healthy individuals, correlating with the disease severity.<sup>[4,5]</sup> In another study there was not any relation between serum total IgE level and exhaled nitric oxide, result of skin test and also severity of asthma.<sup>[6]</sup>

The primary goal of this survey was to analyze the serum levels of IL-33 in adult

patients with asthma and compare the results with healthy controls. Furthermore, the association of IL-33 serum level and disease severity of asthma was investigated.

### Methods

This case-control study was carried out on 89 adult asthmatic patients referred to Allergy Clinic of University of Medical Sciences of FASA, Iran, during summer 2013. The study protocol was approved by the local Ethics Committee. Asthma was diagnosed based on history and clinical examination and by inclusion of reversible airway obstruction, defined as an increase of forced expiratory volume in 1 s (FEV1) by 12%, 15 min after salbutamol inhalation (400 µg/spacer).<sup>[7]</sup>

The patients with asthma were classified into four groups regarding the disease severity: intermittent, mild persistent, moderate persistent, and severe persistent (Expert Panel Report 3, 2007).<sup>[7]</sup> Patients who suffered from any chronic disease were excluded from the study.

Fifty-seven healthy individuals were selected with no signs of allergic or

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#### Access this article online

**Website:**  
www.ijpvmjournal.net/www.ijpvm.net

**DOI:**  
10.4103/ijpvm.IJPVM\_179\_16

#### Quick Response Code:



**How to cite this article:** Momen T, Ahanchian H, Reisi M, Shamsdin SA, Shahsanai A, Keivanfar M. Comparison of Interleukin-33 serum levels in asthmatic patients with a control group and relation with the severity of the disease. *Int J Prev Med* 2017;8:65.

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inflammatory diseases. After obtaining written informed consents, patients were visited by a physician, and their demographic information, respiratory data, duration of their disease, and concurrent other allergic diseases including allergic rhinitis and atopic dermatitis were recorded in a questionnaire. Both groups of patients and control were age and sex matched.

IL-33 level and total IgE between asthmatic and control groups were compared. Furthermore, the comparison was performed in patients with asthma between different groups regarding the levels of disease severity.

Furthermore, in the asthmatic group, the levels of IL-33 and other measures were compared: age, sex, levels of total IgE, FEV1, duration of disease, and association with allergic rhinitis and atopic dermatitis.

### Measurement of serum levels of interleukin-33 and total IgE

Commercial enzyme-linked immunosorbent assays (ELISA) were applied to measure serum levels of IL-33 (BioLegend, USA). The assay was conducted using the protocols recommended by the manufacturers (standard range: 15.6–1000 pg/ml sensitivity: 4.14 pg/ml).

Level of total IgE was determined using ELISA (Monobind, USA). Levels of total IgE level were regarded as a marker of severity of disease in asthmatic patients. Spirometry was done with a portable spirometer (MIR, Italy) in asthmatic patients. The best FEV1 value was selected for analysis.

### Statistical analysis

For basic comparison of IL-33, total IgE, and age between two groups of patients and control and also in asthmatic group for comparison of level of IL-33 in both sex and in patients with or without allergic rhinitis and eczema, independent *t*-test was used. The relationship between IL-33 with age, IgE, and disease duration and also the relation of FEV1 with IL-33 and IgE in asthmatic patients were analyzed by bivariate correlation and Pearson coefficient. For comparison levels of IL-33 and total IgE in different severity groups of asthma, one-way ANOVA test was used. IL-33 and IgE were normalized by one-sample Kolmogorov–Smirnov test. Significant level was considered as  $P < 0.05$ . Data were analyzed using SPSS software version 16 (SPSS Inc., Chicago, USA).

### Results

In this study, 89 asthmatics (49 women and 40 men, with a mean age of  $41 \pm 14.5$  years [age range: 22–65]) and 57 nonasthmatics (34 women and 23 men, age range of 18–67, and mean age  $41 \pm 15$  years) were examined. Table 1 shows the characteristics of nonasthmatic and asthmatic patients and the comparisons of mean age, IL-33, total IgE level, and sex ratio between the groups.

Higher levels of IL-33 were detected in asthmatic patients compared with controls ( $322.6 \pm 241$  vs.  $139.7 \pm 68.1$  pg/ml;  $P = 0.0001$ ). Furthermore, the patients with asthma had a significantly higher level of total IgE than healthy control ( $169.8 \pm 153$  vs.  $108.42 \pm 118$  IU/ml;  $P = 0.008$ ).

The correlation between IL-33 and total IgE revealed significantly direct associations between them ( $P = 0.003$ ,  $R = 0.31$ ). Furthermore, the association of IL-33 with age showed that older age asthmatics had significantly higher levels of IL-33 ( $P = 0.02$ ,  $R = 0.23$ ), but there was not any association with disease duration and IL-33 level [Table 2]. The level of IL-33 did not show significant differences between both sex, and in the patients with or without allergic rhinitis and atopic dermatitis [Table 3].

In the asthmatic patients, 21 intermittent, 28 mild persistent, 21 moderate persistent, and 20 severe persistent patients

**Table 1: Patients' demographics**

Groups	Controls (n=57)	Asthmatics (n=90)	P
Age (years), mean±SD	41±15	41±14.5	0.97**
Sex (male/female)	23/34	40/49	0.58*
Serum IL-33 levels (pg/ml), mean±SD	139.7±68.17	322.6±241	0.0001**
Serum total IgE level (IU/ml), mean±SD	108.42±118.85	169.8±153.5	0.008**

\*Chi-square, \*\*Independent *t*-test. n=Number, SD=Standard deviation, IL=Interleukin-33

**Table 2: Association of interleukin-33 level with age, total IgE level, and disease duration**

Parameter	P	Pearson correlation (r)
Age	0.02	0.23
Total IgE	0.003	0.31
Disease duration	0.051	0.2

**Table 3: Comparison association of the mean level interleukin-33 level in both sex and in patients with or without allergic rhinitis and atopic dermatitis with sex, presence or absence of allergic rhinitis, and atopic dermatitis**

	Serum IL-33 levels (pg/ml), mean±SD	P*
Sex		
Male	344.75±243.79	0.67
Female	322.73±240.77	
Allergic rhinitis		
Positive	371.29±236.75	0.058
Negative	269.67±238.09	
Atopic dermatitis		
Positive	337.15±244.8	0.86
Negative	325±224	

\*Independent *t*-test. SD=Standard deviation, IL=Interleukin-33

entered the study. The mean IL-33 levels and total IgE levels between different groups of asthma were compared in terms of disease severity. The results showed that the mean level of IL-33 and total IgE had statistically significant differences between these groups [Table 4 and Figure 1].

In asthmatic patients, there was a significant negative correlation between FEV1 and total IgE ( $P = 0.028$ ,  $R = -0.23$ ). In addition, a significant negative correlation was observed between FEV1 and IL-33 ( $P = 0.0001$ ,  $R = -0.83$ ).

### Discussion

The relation of IL-33 with asthma and allergies is still uncertain. Several studies on animals, applying recombinant protein to leukocyte cultures, support the role of IL-33 in promotion of Th2-type immune responses; however, only a few studies have been conducted in humans.<sup>[8]</sup> The impact of IL-33 has been proved in some allergic diseases such as anaphylaxis, atopic dermatitis, allergic rhinitis, and allergic conjunctivitis.<sup>[9-12]</sup>

A number of candidate genes have identified by genome-wide association studies that contribute to asthma. Recently, studies suggested that variation in genes encoding IL-33 and IL-1 receptor-like 1 (IL-1RL1) has association with asthma. IL-1RL1 is a part of the IL-33 receptor complex.<sup>[13]</sup> IL-33 is a member in IL-1 family of cytokines such as IL-1 $\beta$  and IL-18, but IL-33 promotes Th2 cells, unlike other members which mostly create TH1 inflammation.<sup>[14,15]</sup> After epithelial cell injury, IL33 releases

as an alarm signal and activates other immune cells such as basophils, Th2 cells and mast cells, leading to the secretion of other cytokines like as IL5 and IL13 which have major role in starting allergic inflammation in asthma.<sup>[16]</sup>

In the current study, a significant rise was reported in serum levels of IL-33 in the asthmatic patients compared with the healthy participants. Furthermore, higher serum IL-33 levels were correlated with disease severity because the serum from moderate and severe asthmatic patients indicated a significant strong trend of IL-33 serum levels and negative correlation between IL-33 and FEV1.

Some studies showed higher levels of IL-33 in asthmatic patients and propose its relationship with disease severity, as one survey showed elevated expression of IL-33 in the epithelial cells and on bronchoalveolar lavage fluid of 25 cases with asthma against to healthy controls. Furthermore, this study found a direct correlation between higher levels of IL-33 and severity of asthma.<sup>[5]</sup> Hamzaoui *et al.* showed higher levels of IL-33 in the serum and sputum of 37 asthmatic children compared with controls.<sup>[17]</sup> Another study revealed significant increased levels of IL-33 in 30 asthmatic patients (15 stable asthma and 15 asthma patients in exacerbation subjects), particularly in the exacerbation group.<sup>[18]</sup> In another article there was not any relation between serum total IgE level and exhaled nitric oxide, result of skin test and also severity of asthma.<sup>[6]</sup>

The current study revealed a direct relationship between IL-33 serum levels and total IgE levels. Many studies confirm the relationship of total IgE and the severity of asthma. IL-33 stimulates B-cell expansion and IgE synthesis causing IL-4 secretions by innate cells. Simultaneously IL4 triggers production of total serum IgE by promoting interaction of CD40 on B-cells and CD40 ligand on T-cells. Thus, IL-33 may have a role in all IgE-mediated allergic diseases.<sup>[19]</sup>

In this work, a larger sample of participants with asthma was examined compared with the previous studies. A cross-sectional study was conducted which is considered as a limitation.

Growing evidence has proposed IL-33 a novel therapeutic target for allergic diseases such as asthma. Recent studies have shown beneficial effects of the IL-33 antagonist in murine models of allergic rhinitis, lower airway inflammation, and allergic contact dermatitis, suggesting IL-33 a potential therapeutic target against allergies.<sup>[20-22]</sup>

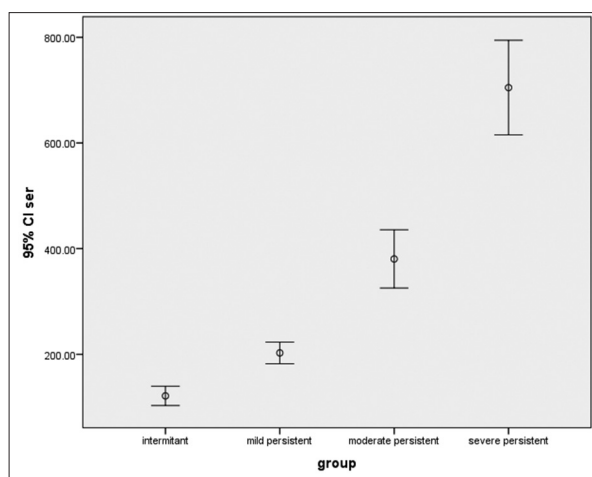


Figure 1: Mean values of interleukin 33 (ng/ml). In different groups of asthma according to severity assessment

**Table 4: Comparison the mean levels of interleukin-33 and total IgE with severity classification in asthmatic patients**

Severity classification	Mild intermittent (n=21)	Mild persistent (n=28)	Moderate persistent (n=21)	Severe persistent (n=20)	P*
Serum IL-33 level (pg/ml)	121.3 (103-139.7)	202.6 (182.1-223.2)	380.4 (325.3-435.5)	704.7 (615.1-794.2)	0.0001
Serum total IgE level (IU/ml)	170.4 (98.7-242)	118.6 (69.2-168)	164.1 (94-234.2)	250 (173-328.5)	0.035

\*One-way ANOVA. *Post hoc* test for IL-33 is significant between all groups. *Post hoc* test for IgE is significant between severe asthma and other groups. IL-33=Interleukin-33, n=Number

Some patients with severe refractory asthma respond poorly to high-dose inhaled or systemic glucocorticoid treatment.<sup>[23]</sup> The mechanisms regarding the inadequate control of symptoms are poorly understood. The majority of biologics are inadequate regarding the clinical setting in asthma.<sup>[24]</sup> Finding a target as a central trigger of inflammation in asthma is effective to develop novel treatment strategies against IgE-mediated allergies. IL-33 is possibly such a molecule, acting early in the allergic cascade following epithelial damage in response to different environmental stimuli or cellular damage. It recruits and activates the cells responsible for the disease, indicating a fundamental role in the pathophysiology of asthma.<sup>[24,25]</sup>

It was speculated that IL-33 elevation is responsible for the maintenance of airway inflammation and hypersensitivity, especially in severe asthmatic cases.

## Conclusions

The results approve the role of IL-33 in the pathogenesis of airway inflammation and remodeling in severe and poorly controlled asthmatic patients. Further studies are required to prove the role of this cytokine as a new therapeutic target in asthma and other allergic diseases.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

**Received:** 15 May 16 **Accepted:** 06 Mar 17

**Published:** 31 Aug 17

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