

## Serum interleukins 6 and 8 in mild and severe asthmatic patients, is it difference?

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### Abstract

**Background:** About 5–10% of patients with asthma suffer from poorly-controlled disease despite corticosteroid (CS) therapy.

**Methods:** 21 severe and 30 mild asthma patients were recruited and underwent collection of blood sample. We determined whether there were any differences in inflammatory biomarkers between severe and mild asthma patients or not.

**Results:** Levels of Interleukin-8 (IL-8) and Interleukin-6 (IL-6) in blood supernatants were similar in both groups of asthma patients. Leukocytes were in range of normal in all patients. Increased eosinophil was in 29% of severe cases and 23% in mild cases. IgE level was increased in 43% of severe form and 50% in mild form.

**Conclusion:** There is not any difference between severe and mild asthma in serum IL-8 and IL-6. Therefore, level of serum cytokines may not predict severity of asthma.

**Key words:** Severe asthma, Mild asthma, Interleukin 6, Interleukin8.

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Allergic asthma is a complex and heterogeneous disease that is characterized by intermittent reversible obstruction and chronic inflammation of the airways, bronchial hyperactivity and an infiltration of lymphocytes and eosinophils into the airway submucosa (1). It has been shown that the integration of Thelper (Th) cells, mast cells and basophils plays an important role of bronchial asthma (2). Interleukin-17 (IL-17) is another novel proinflammatory Th1 cytokine produced by activated T helper cells (3). It is capable of inducing the production of pro-inflammatory cytokine IL-6 and Granulocyte Macrophage- Colony Stimulating Factor (GM-CSF), prostaglandin E2, leukaemia inhibitory factor and intercellular adhesion molecule-1, proliferation of T cells as well as growth and differentiation of CD341 human progenitors into neutrophils (4, 5).

These patients with severe asthma suffer greater morbidity, face a higher risk of asthma death and consume a greater proportion of health resources than other asthmatic patients. One of the hypotheses about severe asthma is that patients may present with an enhanced inflammatory response, or that the characteristic eosinophilic inflammation in the airways of asthma is replaced by other types of cellular inflammation.

The American Thoracic Society's (ATS) and European Respiratory Society workshop definition requires the presence of at least two of seven different minor criteria, despite the use of high-dose inhaled corticosteroid or the long-term use of oral steroids (6, 7).

To further investigate the role of proinflammatory and Th cytokines in the pathogenesis of allergic asthma, we measured the plasma concentrations of two proinflammatory cytokines IL-6 and IL-8, in patients with severe asthma. We compared these findings with those from patients with mild asthma.

## Methods

Asthma was diagnosed by a physician. Current and ex-smokers of 45 pack-years were excluded. According to previous studies, we selected 51 asthmatic patients. Severe (n=21) and mild (n=30) asthmatics were defined according to guidelines developed by the Severe Asthma Research Program based on ATS and GINA criteria (3). They had either one or two major criteria for corticosteroid usage, and had three or more minor criteria with 13 having five or more. Patients who did not meet the criteria for mild asthma were classified as mild asthmatics (n=30). All participants gave informed consent to a protocol approved by the Ethics Committee of the local center.

Before introducing any drugs such as systemic corticosteroids (new cases), assay of interleukin-8 and interleukin-13, the blood supernatants (5cc blood vein sample) were assayed for IL-6 and IL-8 levels by Elisa (Diacolon, France) following the manufacturers' instructions, with a lower detection limit of 2 and 29 pg/mL, respectively. All of blood supernatants were collected in -70 °C before assay. Total IgE was assayed by Elisa (EU-immune, Germany) with lower limit 100 IU/ml. Complete Blood Cell Count (CBC) with differentiation was assayed for all of them. All data recorded in SPSS-17 and analyzed quantities' data with t-test and qualities` data with X<sup>2</sup> test.

Data are presented as mean±SD. A P-value of 0.05 with 95% confidence index was accepted as significant.

## Results

A comparison of the baseline data of patients with severe and non-severe asthma is presented in Table 1. The mean of age of the patients was 41±15 years (range 10-50 yr). Characteristics of the 21 subjects with severe asthma and 30 with mild or treatment responsive asthma are provided in Table 2. Serum levels of IL-6 in severe and mild asthma patients were 2.8 vs. 3.19 and IL-8 22.2 vs. 30.54 pg/mg, respectively, without any statistical significance (table 4).

**Table 1: distribution of severe and mild asthma according gender**

	Gender	Male	Female	Total
<b>Asthma</b>		<b>N(%)</b>	<b>N(%)</b>	<b>N(%)</b>
Severe		13(62)	8(38)	21(41)
Mild		9(30)	21(70)	30(59)
Total		22(43)	29(57)	51(100)

**Table 2: distribution of severe and mild asthma according IgE and eosinophil**

Symptom	Mild Asthma N(%)	Severe Asthma N(%)	Total N(%)	Pvalue
Gough	28(96)	20(91)	48(94)	0.334
Wheezing	14(48)	11(50)	25(49)	0.322
Dyspnea	10(34)	20(91)	30(59)	0.001

**Table 3: distribution of severe and mild asthma according Eosinophil, IgE, IL-6 and IL-8 levels**

	Severe asthma	Mild asthma	Pvalue
IL-6(pg/ml) (Normal<2)	2.8±2.09	3.19±2.19	0.553
IL-8(pg/ml) (Normal<29)	22.2±11.49	30.54±17.20	0.253
Total IgE	251.9±205.75	206.7±171.5	0.410
Eosinophil	670.01±284.10	400.83±128.71	0.119

## Discussion

In this study, patients with severe asthma were compared with patients with mild asthma, we have shown that there are very little detectable differences in the proinflammatory cytokines (IL-6, IL-8) in serum (P> 0.05).

Many studies have suggested that the severity of asthma is related to the degree of inflammation (8, 9). Animal models and clinical studies in humans have indicated an important role for Th2 cells producing IL-4, IL-5, IL-6, IL-8 and IL-13 in the pathogenesis of allergic asthma (1). However, the most significant findings were those related to increase in IL-6 serum levels to normal level (<2pg/ml) in severe asthma (mean=2.8 pg/ml), compared to mild asthmatic (mean=3.19 pg/ml) patients (p< 0.05), even the IL-6 level in mild asthmatic patient were a little more than severe asthmatic patients. Although there was increased level of IL-8 to normal level (<29 pg/ml) in mild asthmatic patients (mean=30.54 pg/ml) but in the severe cases, the level of IL-8 was in normal range (mean=22.2 pg/ml). Circulating levels of TNF-α and IL-6 are reported to have increased in Chronic obstruction pulmonary disease (COPD) patients, and associated with malnutrition in these patients (10). Recent reports have shown that asthma patients also

exhibit systemic inflammation (11,12). Yokoyama et al showed that circulating IL-6 levels were higher in asthma patients compared with control patients, and that IL-6 levels were increased after asthmatic attacks (12). In our study there is not a normal control group, because only we decided to compare between severe and mild asthmatic patients. Patients with severe treatment refractory asthma have a different cytokine profile in bronchial biopsy findings than a group with mild asthma, demonstrating an increase in IL-8 and IFN- $\alpha$ -expressing cells (13). Of course, sometimes there is not any association between serum and bronchial cytokines level. In our study, there is not any significant relation between IgE and eosinophil with increased in cytokines level. Increased in IgE level was done with 6 patients that had increased IL-6 and IL-8 (5 mild and 1 severe). Also there was increase in eosinophil in 6 cases with increasing cytokines (3 mild and 3 severe). Nonetheless, in non increasing cytokines, IgE in 7 cases increased and eosinophil in 18 cases. Therefore, there is no significant difference between two groups (increase and normal level of IL-6, IL-8). Our study showed that there is no difference between male (n=7, 14%) and female (n=10, 20%) in increasing cytokines.

We found that plasma concentration of the cytokines, IL-6 and IL-8, was not significantly elevated in severe asthmatic patients compared to mild asthmatic patients. In conclusion, the present study has demonstrated that proinflammatory cytokines increased in asthmatic patients (relate to normal level) and probability play critical roles in the inflammatory characters of asthma but there is not any significant difference between severe and mild asthma patients, therefore, serum level of cytokines may not be predict severity of asthmatic patients. One of the limitations of this study was that a few cases were contributed. Therefore, it needs another study with more cases.

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