

# Soluble Form of FasL (sFasL) in Adult Asthma

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## Abstract

**Background:** sFasL is the soluble form of FasL inducing apoptosis by binding to Fas. Fas/sFasL could be the most important mechanisms in inflammatory conditions such as asthma by controlling inflammatory responses. This study was undertaken to determine the level of sFasL in allergic and non-allergic asthmatic patients with different stages of asthma control.

**Methods:** Twenty asthmatic patients were enrolled and divided into controlled and uncontrolled patient groups. They were divided into 4 subgroups including controlled/allergic, controlled/non-allergic, uncontrolled/allergic and uncontrolled/non-allergic subgroups. Five normal subjects were selected as a control group. From all subjects, 3 ml of blood was obtained and sFasL and IgE serum levels were evaluated by a specific ELISA kit.

**Results:** sFasL in the controlled and uncontrolled patient groups did not have any significant difference; but in the uncontrolled/allergic subgroup, it was significantly lower than that in the control group and also higher in the uncontrolled/non-allergic subgroup insignificantly.

**Conclusion:** In patients with acute inflammatory conditions, sFasL had an increasing effect to control inflammation observed in uncontrolled/non-allergic subgroup, but unexpectedly not in the uncontrolled/allergic subgroup. Probably in allergic patients, there are factors or mechanisms that inhibit sFasL production or expression.

**Keywords:** Asthma; sFasL; IgE; Allergy

## Introduction

The Fas ligand (FasL) is a 37-kDa type II membrane protein, belonging to the TNF family, which includes TNF, lymphotoxin, TNF-related apoptosis-inducing ligand (TRAIL), CD40 ligand, CD27 ligand, CD30 ligand, and OX40 ligand.<sup>1</sup> FasL is also expressed on activated T cells that have cytotoxic activity and in the immune-privileged tissues such as testis and eyes that eliminate infiltrating immune cells.<sup>1,2</sup>

FasL is a ligand for Fas (also called APO-1 or CD95) and a 45-kDa type I membrane receptor protein which belongs to the tumor necrosis factor and/or nerve growth factor-receptor superfamily, that is ex-

pressed in various tissues such as the thymus, liver, skin, ovary, and lung.<sup>2</sup> Fas and FasL both play a key role in the regulation of apoptosis within the immune system, especially in lymphocyte development, antiviral immune responses, the elimination of tumor cells and inflammatory responses.<sup>3</sup> Binding of FasL to Fas leads to formation of death-inducing signaling complex (FADD and caspase-8) that activates effector caspases, especially caspase-3. Activated caspase-3 cleaves DNA repair enzymes, cellular and nuclear structural proteins, endonucleases, and many other cellular constituents, culminating in cell death.<sup>4</sup> The soluble form of FasL (sFasL) is naturally produced by metalloproteinase-mediated processing and can bind to Fas and initiate the same cascade.<sup>1</sup>

Asthma is a multifactorial chronic inflammatory disorder with two main characteristics, bronchial hyperresponsiveness (BHR) and often enhanced total serum IgE levels and is defined as a respiratory form of allergy.<sup>5</sup>

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It has been shown that Fas/FasL-mediated apoptosis is crucial in prenatal murine<sup>4</sup> and fetal rabbit lung development.<sup>6</sup> Some studies have suggested a correlation between Fas-ligand and allergic disease, and have shown a high expression of Fas-ligand in the activated lymphocytes of allergic patients.<sup>7</sup> FasL has been demonstrated in the air ways' epithelial cells of *gld* FasL mutated mice, which could prevent allergen-induced airway inflammation.<sup>2</sup>

Circulating soluble form of FasL (sFasL) is elevated in the serum of patients with lymphoma,<sup>3</sup> infections,<sup>8,9</sup> autoimmune diseases such as Hashimoto's thyroiditis and Graves' disease<sup>10</sup> and inflammatory disorders.<sup>2,11</sup> sFasL is present in bronchoalveolar lavage (BAL) fluid of patients before and after the onset of acute respiratory distress syndrome (ARDS).<sup>11</sup> In allergic patients, the levels of sFasL were increased during the pollen season.<sup>12</sup> In this study, we evaluated the level of sFasL in allergic and non- allergic asthmatic patients with different stages of asthma control.

**Material and Methods**

From 81 asthmatic patients participating for cytokine polymorphism evaluation, 20 were selected and classified into 2 groups of controlled and uncontrolled ones. Each group was divided into allergic and non-allergic subgroups (Table 1). A questionnaire was provided for each patient. The patients were unrelated and nonsmoking with a mean±SD age of 25±7 years (range=15-42 years). Pregnant women and patients with a history of cigarette smoking were excluded from the study. A usual anti-inflammatory drug was administered to asthmatics for the control of their disease. Long-term controlled medications such as inhaled corticosteroids were included. These medications treated the airway inflammation due to asthma signs and symptoms. In the attack phase, short-acting β2-adrenergic receptor agonist was taken as a phase quick-relief inhaler such as salbutamol. Asthma disease and asthma control levels were diagnosed according to American Thoracic Society (ATS) criteria.<sup>13</sup> Five unrelated normal subjects with normal

spirometric values and no respiratory symptoms were selected as a control group. The study protocol was approved by the ethics committee at our institution, and written informed consent was obtained from all the participants.

From 5 ml of the blood provided from patients and normal subjects, serum was separated and serum levels of sFasL (ng/ml) were determined by a commercial sandwich ELISA kit with the use of monoclonal antibodies (Biosource.USA). The lower limit of detection sensitivity was <20 pg/ml sFasL with a range of 0-20 ng/ml.

Total serum IgE levels were measured by using the ELISA kit (Genesis Diagnostics UK) according to the manufacturer's instructions. The minimum detectable concentration of Total IgE was 0.9 IU/ml and the range of detection was 0-1250 IU/ml.

The results were statistically evaluated, using Statistical Package for the Social Sciences (SPSS; version 15, Chicago, IL, USA), and non-parametric Mann-Whitney test. The results are expressed as mean±standard deviation and range values. Correlations in Spearman's test were done for evaluation of correlation between respirometric factors of patients. A probability value of  $p < 0.05$  was considered statistically significant.

**Results**

The results of different characteristics for the asthmatic subgroups and control group are shown in Table 2. There were no significant differences for sFasL between asthmatic patients and the controls ( $p=0.213$ ); also, there was no significant difference between the controlled ( $p=0.278$ ) and uncontrolled asthmatic groups ( $p=0.272$ ) and the normal controls. In the asthmatic subgroups, sFasL in the uncontrolled/allergic subgroup was significantly lower than that in the controls ( $p=0.027$ ) and in the uncontrolled/non-allergic subgroup it was higher than that in the controls, but not significant ( $p=0.914$ ). In the other subgroups, it was not statistically significant ( $p \geq 0.05$ ). IgE level in the asthmatic patients were

**Table 1:** Subject characteristics

Characteristics	Controls	Asthmatics			
		Controlled		Uncontrolled	
		Allergic	Non allergic	Allergic	Non allergic
Sex / M/F ratio	3/2	2/3	3/2	2/3	3/2
Age / yrs (mean±SD)	24±8	23±8	28±7	22±6	24±7

**Table 2:** Result for sFasL and IgE serum level and pulmonary function tests for asthmatic patients and controls.

Characteristics	Controls	Asthma	Asthmatics			
			Controlled		Uncontrolled	
			Allergic	Non allergic	Allergic	Non allergic
sFasL (ng/ml)	0.180±0.014	0.170±0.022	0.171±0.019	0.173±0.013	0.152±0.015	0.188±0.030
IgE (IU/ml)	50.2±32	287.2±407	808.2±547	41.6±70	251.7±24	47.3±62
FEV1* (% pre-dicted)	87.3±14	80.4±22	99.1±15	79.4±9	61.9±28	81.6±26
FEV1/FVC <sup>+</sup>	88.1±11	84.1±8	89.1±9	87.5±7	76.6±7	85.1±10
PFE <sup>#</sup>	91.6±21	73.5±23	81.8±21	74.6±25	60.9±32	75.5±25
FEF.sub.25-75 <sup>&gt;</sup>	84.7±17	63.2±35	70.9±4	68.8±38	50.6±20	61.5±45
FVC (L/% pre-dicted)	93.4±8	78.9±23	98.5±24	78.7±6	69.6±30	71.2±28

\*FEV1: Forced Expiratory Volume in 1 Second, <sup>+</sup>FVC: Forced Vital Capacity, <sup>#</sup>PEF: Peak Expiratory Flow, <sup>></sup>FEF.sub.25-75: Forced Expiratory Flow 25-75% or 25-50%

significantly higher than that in the controls ( $p=0.043$ ). IgE serum levels in the allergic patients were higher than those in the non-allergic asthmatics with no statistical significance ( $p=0.144$ ). There was a statistically significant correlation between spirometric factors in the asthmatic patients ( $p<0.05$ ).

The IgE level in asthmatic patients was significantly higher than that in the controls ( $p=0.043$ ). sFasL in the uncontrolled/allergic subgroup was lower than that in the controls ( $p=0.027$ ).

## Discussion

The Fas/FasL system is thought to be involved in T-cell cytotoxicity functions, modulation of the inflammatory response, maintenance of the immune privileged status of the eye and testis, tumor evasion from host immune response, and regulation of turnover in certain epithelial cell populations.<sup>1,2</sup> The sFasL could be a component in the regulation of apoptosis during lymphocyte development; cell mediated immunological control of virus infection, and tumor growth.<sup>3</sup>

The role and molecular function of the Fas/FasL system in the immune system has been extensively studied and various results were presented. Some reports showed that in addition to inducing cell death, Fas can mediate other activation signals including increased proliferation in human T cells and fibroblasts, induction of IL-8 synthesis in human colon carcinoma cells, and activation of the transcription factor NF- $\kappa$ B.<sup>14</sup>

sFasL shed from activated T cells exhibited a potent chemotactic activity against both human and mouse PMNs<sup>1</sup> and in the normal human diploid cell

line GM6112, sFasL leads to morphological signs of cell death in less than 1% of the cells.<sup>14</sup>

These observations indicate that Fas-mediated signaling is not limited to inducing cell death. Even, it has been shown that sFasL inhibits the killing of fresh peripheral blood T lymphocyte by membrane FasL, and shedding of FasL from the membrane is a mechanism for downregulating at least part of its killing activity.<sup>15</sup> In heart transplantation such an inflammatory response changes in sFas and sFasL levels in the stable recipients and in the patients experiencing acute rejection.<sup>16</sup> In the case of asthma and allergy, also results are variable. It was reported that FasL increased in activated lymphocyte patients with allergic disease;<sup>7</sup> or sFasL levels in asthmatic patients that had received inhaler corticosteroid (ICS) treatment were higher than those in patients who did not receive ICS treatment during the acute attack.<sup>17</sup> Another study showed that sFasL could not maintain inflammatory responses induced by allergens that cause mucous cell metaplasia (MCM) airway obstruction in asthmatics.<sup>18</sup>

In this study, we evaluated the levels of sFasL as reflection of immune system response for control of inflammation in allergic and non-allergic asthmatic patients with different stages of asthma control. Our study demonstrated that there were no significant differences in sFasL between the two groups of controlled asthma patients and uncontrolled patients, compared with normal control group.

In the case of controlled group, sFasL in 2 allergic and non-allergic subgroups were almost similar to that in the control group. These results are in the same line with most of the findings and suggest that in the stable phase of asthma like normal subjects there are

not any Fas stimulation and release of sFasL.

In case of uncontrolled groups, sFasL in the allergic subgroup was significantly lower than that in the control and in the non-allergic subgroup, it was higher than that in the control (but was not significant). These results suggest that in non-allergic patients, sFasL could be released to control inflammatory responses, and it is a regulatory factor of the immune system. Significant decrease of sFasL in allergic/uncontrolled patients raises a question that why in the patients with acute inflammation sFasL is decreased. Some studies demonstrated an increase of sFasL in allergic asthma patients but these increases were associated with the type of anti-inflammatory therapy<sup>17</sup> or type of allergen in seasonal allergy.<sup>12</sup> So, several factors could affect serum sFasL. Maybe in our allergic patients there were factors or mechanisms that inhibited production, increased consumption or

resulted into degeneration of sFasL.

Probably in allergic patients, there are factors or mechanisms that inhibit sFasL production or expression. Although in this study we studied the probable role of sFasL as a regulatory molecule in asthma, there are many questions and drawbacks. Further studies with higher sample size and consideration of other regulatory molecules or interference of other factors such as seasonal factors will be needed for more clarifications.

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**Conflict of interest:** None declared.

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