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# Soluble trail as a marker of efficacy of allergen-specific immunotherapy in patients with allergic rhinoconjunctivitis

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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## Summary

**Background:** Allergic rhinitis is a common health problem affecting the immune system. The homeostasis of the immune system is regulated by apoptosis. In this study, serum circulating soluble TRAIL levels of allergic rhinoconjunctivitis patients before and after allergen-specific immunotherapy were evaluated.

**Material/Methods:** The sTRAIL levels of pre- and post-treated allergic rhinoconjunctivitis patients (n=25) were compared to age- and sex-matched healthy individuals (n=25). sTRAIL levels were measured by ELISA. The skin prick test (SPT) results were recorded before and after treatment.

**Results:** The sTRAIL levels between the pre-treated and control groups were significantly different ($p<0.0001$). However, there was no significant difference between the post-treated group and healthy individuals ($p=0,801$). SPT was a statistically significant difference between the values of the research group before and after immunotherapy (grasses mixture, barley mixture, Oleaauropeae, D. Pteronyssinus, D. farinae).

**Conclusions:** The sTRAIL levels were decreased after allergen-specific immunotherapy to healthy levels and may be of use as a marker of efficacy of immunotherapy in allergic rhinoconjunctivitis patients.

**key words:** **allergen-specific immunotherapy • sTRAIL levels • allergic rhinitis • skin prick test**

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## Background

Allergic rhinitis is a common health problem and has 2 forms seasonal and perennial. The prevalences of asthma, allergic rhinitis and allergic eye disease in Antalya, on the south coast of Turkey, have been reported as 8.2%, 10.8% and 7.5%, respectively [1,2]. Allergic diseases are most likely due to complex interactions between largely unknown genetic and environmental factors [3–6]. The micro-array techniques for the detection of specific IgE have improved the diagnostic procedures for allergic diseases. This method also allows definition of sensitization profiles from an epidemiological point of view [5].

A detailed knowledge of the sensitization pattern may have relevant implications for the prescription of specific immunotherapy. A number of epidemiologic studies have also supported a relationship between allergic rhinoconjunctivitis and diet, hygiene, and life-style, suggesting that environmental factors also impact the development of allergic rhinoconjunctivitis. Apoptosis is an active physiological process that can cause an inflammatory reaction and tissue damage, and is fundamental to maturation and homeostasis in the immune system [7]. It can be induced passively, through lack of essential survival signals, or actively, through ligand-induced trimerization of specific death receptors of the tumor necrosis factor (TNF) receptor family, such as Fas, the TNF receptor, or the TNF-related apoptosis-inducing ligand (TRAIL) receptor [8]. TRAIL also is able to prevent apoptosis through the actions of its decoy receptors, DcR-1 and DcR-2. Various regulators of TRAIL include FADD, IAPs, Bcl-2s, p53, and FLIPs. TRAIL is present in cells involved in asthma, including eosinophils, mast cells, fibroblasts, and airway epithelial cells. It is expressed in airway remodeling and may be linked with the pathways of transforming growth factor beta1, which is thought to cause damage to the epithelium. The repair process of the epithelium is hindered as a result of increased apoptosis induced by TGF-beta1, which overlaps with the pathways of TRAIL. Analogs of TRAIL could have therapeutical applications for asthma. TRAIL is also seen as the basis for a "miracle" drug for cancer because of its ability to selectively kill cancer cells [9]. It has previously been reported that negative selection of T cells in the thymus is controlled by TRAIL [10]. For example, mice deficient in TRAIL had a severe defect in thymic deletion of T cells and were hypersensitive to collagen-induced arthritis [11].

Mast cells activation through Fc epsilon RI cross-linking has a pivotal role in the initiation of allergic reactions. IgE-dependent activation increases TRAIL-induced caspase-8 and caspase-3 cleavage, and regulates human mast cell apoptosis by fine-tuning anti-apoptotic and pro-apoptotic factors [12].

Our study aimed to identify the role of sTRAIL in the pathophysiology of allergic rhinoconjunctivitis, and to explore whether allergen-specific subcutaneous immunotherapy treatment of allergic rhinoconjunctivitis patients altered any observed effect of sTRAIL.

## Material and Methods

### Patients

The study was conducted in Antalya, Turkey between 9 January 2009 and 28 January 2010. The study was approved by the local ethics committee, and written consent was obtained from all patients and healthy volunteers. All patients were followed in the Immunology and Allergy Clinic of Antalya Education and Research Hospital. Subjects with kidney disease, heart disease, liver disease, diabetes mellitus, cancer status, obesity, (body mass index (BMI) ⩾30 kg·m$^2$), and autoimmune disease were excluded clinically and serologically.

The first group of 25 patients included 11 male and 14 female subjects with allergic rhinoconjunctivitis, having a combined mean age of 38.56±12.03 years. The first group included 2 measurements in the same patients; group-IA represents data recorded before subcutaneous allergen-specific immunotherapy, and group-IB shows data recorded 12 months after the subcutaneous allergen-specific immunotherapy. All patients received immunotherapy every 4 weeks. The symptoms and severity of allergic reactions were recorded before and after treatment.

Assessment of clinical changes and adverse effects were recorded at regular follow-up. The total duration of allergic rhinitis was 8.1±3.2 years. A second group of 25 healthy individuals (11 male and 14 female) (Group II) had a mean age of 38.23±12.21 years (Table 1).

Subjects with kidney disease, heart disease, liver disease, diabetes mellitus, cancer status, obesity (body mass index (BMI) ⩾30 kg·m$^2$), and autoimmune disease were excluded clinically and serologically.

### Laboratory investigations

Blood samples were collected into 5 mL plain Vacutainer tubes and centrifuged at 3000 × g for 10 min. Serum IgE levels, hepatitis markers (HBs Ag, Anti HBs, Anti HCV) were evaluated in all patients. Total and specific IgE levels were enumerated by fluoroenzyme immunoassay (ImmunoCAP-FEIA) using an ImmunoCAP kit (Phar-macia, Uppsala, Sweden). Values above 100 kU/L and 0.35 kU/L for total and specific IgE levels were considered abnormal.

**Table 1.** Demographics for allergic rhinitis patients (group I) and healthy control group (group II).

| Group number | Age (years) | Gender (female/ male) | Duration of allergic rhinitis symptoms (years) | Skin test sensitiviy | Smoker (yes/no) | BMI |
|---|---|---|---|---|---|---|
| Group I (n=25) | 38.56±12.03 | 14/11 | 8.1±3.2 | Grass, wheat, trees (olive), mite | 6/19 | 26.7±4.8 |
| Group II (n=25) | 38.23±12.21 | 14/11 | – | – | 8/17 | 24.2±3.9 |

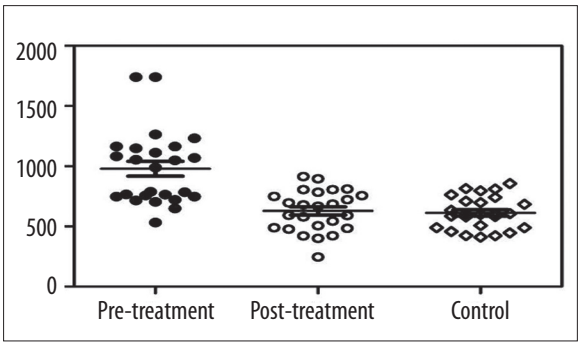


**Figure 1.** The s TRAIL levels of allergic rhinoconjunctivitis patients before (Group IA) and after the subcutaneous immunotherapy (Group IB) and healthy individuals Group II). The sTRAIL (pg/mL) levels of allergic rhinoconjunctivitis patients before (Group IA) and after the subcutaneous immunotherapy (Group IB) and healthy individuals Group II). 85×73 mm (300×300 DPI).

Serum sTRAIL levels in all individuals (patients and healthy controls) were measured by a sandwich enzyme-linked immunosorbent assay (Diaclone, France). All assays were performed in duplicate.

**Skin-Prick Test (SPT)**

The skin prick test results were recorded before and after treatment. Skin prick tests on the forearm were performed in all patients, using standardized latex extract containing high ammonia natural rubber latex, and a full set of 10 common allergens. In addition, venom SPT was performed on 1 patient based on the subject's clinical history. SPTs were performed by skilled nursing personnel. Positive tests were counted as wheals of 3 mm in diameter after 20 min. Test resultss were compared with positive histamine controls and negative saline controls. Commercial extracts used were manufactured by Allergopharma Nova Helisen- German. No intradermal tests were performed.

**Statistical analysis**

The results of patients in both groups were compared with those of healthy subjects. Data were analyzed using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Student's T test was used for comparison of controls and patient groups (group IA and group IB). Paired Samples T test was used for comparison of group IA and group IB.

## Results

**Laboratory findings**

In this clinical follow-up study, 25 patients were already receiving subcutaneous immunotherapy, and these subjects were included for further analysis at our clinic. The mean IgE levels were: Group IA – 699.505 IU/mL; Group IB – 164.115 IU/mL; and Group II – 41.08 IU/mL. There was a statistically significant difference between the values

**Table 2.** While there was a statistically significant difference between pre-treated and control group (p<0.0001), no difference between post-treated and control groups (p=0.801).

| Group | sTRAIL (pg/ml) |
|---|---|
| Group IA: Before subcutaneous immunotherapy (n=25) | 939.85±352.52 |
| Group IB: After subcutaneous immunotherapy (After a year) (n=25) | 628.93±170.5 |
| Group II: Healthy control (n=25) | 612.64±135.6 |

**Table 3.** While there was a statistically significant difference between pre-treated and post-treated group (Grasses mixture, Barley mixture, Oleaauropeae, D. Pteronyssinus, D. farinae).

| Skin prick test | Before subcutaneous immunotherapy | After subcutaneous immunotherapy (After a year) | p |
|---|---|---|---|
| | X±Sd | X±Sd | |
| Positive control | 10.44±3.73 | 10.36±4.10 | 0.792 |
| Negative control | 2.88±1.88 | 1.92±2.08 | 0.095 |
| Grasses mixture | 13.68±4.21 | 7.36±4.64 | 0.000 |
| Barley mixture | 10.16±2.56 | 8.04±2.92 | 0.015 |
| Weed mixture | 4.08±2.91 | 4.16±2.82 | 0.828 |
| Trees mixture | 7.92±4.65 | 6.40±3.71 | 0.333 |
| Oleaauropeae | 11.56±3.19 | 6.68±4.06 | 0.000 |
| D. Pteronyssinus | 7.96±4.64 | 4.00±3.07 | 0.002 |
| D. farinae | 7.08±3.93 | 3.52±3.23 | 0.001 |

of the research group before and after immunotherapy (p=0/0005). Prick tests in all patients in Group I were detected in mite, olive and grass allergy. These results correlated with specific IgE, and hepatitis markers were negative in all patients.

As shown in Figure 1, significant difference was seen in the mean values of sTRAIL in allergic rhinitis patients before immunotherapy (n=25; 939.85±352.52 pg/mL), afterc immunotherapy (n=25; 628.93±170.5 pg/mL) and healthy controls (n=25; 612.64±135.6 pg/mL). There was a statistically significant difference between the values of the research group before and after immunotherapy (p=0.001). While there was a statistically significant difference between the pre-treated group and control group (p<0.0001), there was no difference between the post-treated group and healthy individuals (p=0.801). sTRAIL levels of each group are given in Table 2.

**Skin-Prick Test (SPT)**

As shown in Table 3, there was a statistically significant difference between the values of the research group before and after immunotherapy (Grasses mixture, Barley mixture, Oleaauropeae, D. Pteronyssinus, D. farinae)

## Discussion

Allergen-specific immunotherapy has been used in the management of allergic diseases for nearly 100 years. The quality of allergen products is a key issue for both diagnosis and therapy. The results of our previous study suggest that in our region 51.8% of the allergens determined by prick test are mite and 42.3% are pollens. Pollen positivity rate of the cases that had immune-therapy was 61.8%, and mite positivity rate was 60.4%. Most of the cases had rhinitis symptoms due to pollens and mite. Allergens are known to exhibit regional variation in expression, which suggests that the allergen profiles and skin prick tests should be designed with reference to individual locales [2]. Allergen-specific immunotherapy treatment significantly reduced the nasal symptom score across all group I patients studied. In this study, SPT was a statistically significant difference between the values of the research group before and after allergen-specific subcutaneous immunotherapy (Grasses mixture, Barley mixture, Oleaauropeae, D. Pteronyssinus, D. farinae).

Our earlier studies provided a novel perspective on severe persistent allergic asthma and the effect of anti-IgE treatment, using as markers serum soluble TNF-related apoptosis-inducing ligand, total antioxidant capacity, hydrogen peroxide, malondialdehyde, ceruloplasmine oxidase activity, high sensitive C reactive protein and total nitric oxide concentrations measurements [13–16]. In our previous study we found that the TRAIL levels in variances of the patients who had the effective anti IgE treatment were significantly lower than the healthy controls [16].

Engagement of the Fas/FasL system has not yet been shown to contribute to increase apoptosis. However, the importance of the other death pathways is still unknown. Recent interest has focused on the molecule TRAIL, which is involved in the pathophysiology of different diseases, including cancer, diabetes mellitus, autoimmune diseases, and inflammation [17–21]. TRAIL also present in cells that involved in asthma including eosinophils, mast cells, fibroblasts, and airway epithelial cells. It is expressed in airway remodeling and may be linked with the pathways of transforming growth factor-beta (TGF-β), which is thought to cause damage to the epithelium. The repair process of the epithelium is hindered as a result of increased apoptosis induced by TGF-beta, which overlaps with the pathways of TRAIL. Moreover analogs of TRAIL could have therapeutical applications for asthma [22,23]. These results reflect the different mechanism(s) in the pathogenesis of allergic diseases by the regulation of apoptosis.

Desloratadine (DCL) is a non-sedating antihistamine approved for the treatment of allergic rhinitis. Patients in groups I-A and B had a DCL usage history during the exacerbation phase from May to November. Blood samples from the patients were obtained in January, when they would be expected to have fewer allergic symptoms. Mast cells (MC) play a key role in allergy and are involved in several chronic inflammatory diseases. Furthermore, they are involved in innate immunity and in tissue repair [24–27]. MC hyperplasia is observed in certain disease states [26]. The regulation of MC numbers, as of any other normal cells, depends on both their generation rate and survival time within tissues. Many factors regulate MC viability [24]. The critical event in allergic reactions is allergen-induced crosslinking of specific IgE molecules bound to Fc_RI receptors on the MC surface, which triggers MC degranulation and release of inflammatory mediators. Non-IgE-mediated activation may also contribute to continued degranulation of MC during the late phase of allergic reactions [24]. Allergic mechanism are involved in the increased susceptibility of human MC to TRAIL-induced apoptosis after IgE-dependent activation [25]. In this study, IgE levels had a statistically significant difference between the values of the research group before and after allergen-specific subcutaneous immunotherapy.

## Conclusions

Taken together, our results and those of others, suggest that characterization of the specific receptor systems activated, and the pro-inflammatory factors regulated, by TRAIL *in vivo* may lead to the development of novel therapeutic strategies for diseases as diverse as infection, autoimmunity, and allergy.

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