

Evaluation of efficacy of immunotherapy in children with asthma monosensitized to *Alternaria*

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SUMMARY: Kılıç M, Altıntaş DU, Yılmaz M, Bingöl-Karakoç G, Burgut R, Güneşer-Kendirli S. Evaluation of efficacy of immunotherapy in children with asthma monosensitized to *Alternaria*. Turk J Pediatr 2011; 53: 285-294.

In this study, we aimed to evaluate the efficacy of specific immunotherapy (SIT) in children monosensitized to *Alternaria*.

Sixteen children with bronchial asthma monosensitized to *Alternaria* were enrolled in the study. Patients were divided into two groups as the immunotherapy group (Group I; 9 patients) and control group (Group II; 7 patients).

A significant reduction in bronchial responsiveness to methacholine and *Alternaria* was found in Group I after one year of SIT ($p=0.03$, $p=0.006$) in comparison to controls. Specific IgE levels were decreased in the immunotherapy group ($p=0.001$). Following allergen provocation, a rise in sputum eosinophil count was found to be lower in the SIT group compared to controls after one year ($p=0.011$), and sputum eosinophil cationic protein (ECP) levels did not change in the SIT group, while there was a statistically significant increase in controls.

Our results demonstrated that SIT with *Alternaria* caused clear changes in airway responsiveness and serum-specific IgE levels. However, further long-term studies in large series should be carried out for clinical documentation of the efficacy of SIT in the treatment of children with *Alternaria* allergy.

Key words: *Alternaria*, asthma, bronchial hyperresponsiveness, children, immunotherapy.

Various studies have demonstrated that inhalant fungal allergens induce the development of allergic diseases. Significant fungi responsible for allergic diseases, including asthma and rhinitis, are *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*. Environmental fungi are particularly important allergens and, among these, *Alternaria alternata* is thought to cause many of the allergic diseases prevalent in the world¹⁻³.

In contrast to other allergens, the role of allergen-specific immunotherapy (SIT) has been investigated in only a minority of patients with respiratory allergies caused by fungal spores. However, the effectiveness of SIT has been demonstrated in a small number of placebo-controlled studies conducted with extracts of *Alternaria* and *Cladosporium*²⁻⁵. Successful SIT reduces symptoms of allergic disease and

need for medication. It has also been shown to prevent onset of new sensitizations and reduce development of asthma in patients with rhinitis caused by inhalant allergens^{6,7}.

The purpose of this current study was to evaluate the efficacy of SIT in children aged 7-15 years who were monosensitized to *Alternaria*. The first year data of an ongoing five-year study are presented.

Material and Methods

Subjects

The study was a prospective, open parallel-group, controlled study. Twenty-four children aged 7-15 years who were under follow-up in the Department of Pediatric Allergy and Immunology were included. The inclusion criteria were defined as follows: a) a diagnosis

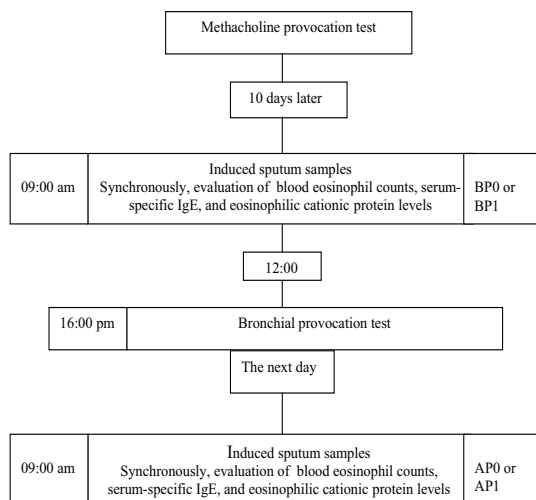


Fig. 1. Design of the study.

BP0: The results before the start of allergen bronchial provocation test at the beginning of the study.

AP0: The results 17 h after the start of allergen bronchial provocation test at the beginning of the study.

BP1: The results before the start of allergen bronchial provocation test after 12 months of the study.

AP1: The results 17 h after the start of allergen bronchial provocation test after 12 months of the study.

of mild to moderate persistent asthma, b) allergy only to *Alternaria*, c) no previous history of immunotherapy, and d) full control cannot be achieved despite receiving medical therapy and optimal allergen avoidance. This study was planned for five years, and in this report, the results of the first year (November 2006 – October 2007) are given. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) guidelines⁸. The patients were randomly divided into two groups. Group I included 12 patients who were started on SIT, and Group II included 12 patients without SIT. Eight patients were excluded during the first year of the study period following withdrawal of parental consent (3 patients in Group I, 5 patients in Group II). A routine skin prick test was performed in all patients using kits containing common inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, grass mix, tree mix, mold mix, *Alternaria* species, *Cladosporium* species, eucalyptus, olive, and cat-dog dander) (ALK-Abelló, Madrid, Spain), and an induration with a diameter of ≥ 3 mm was accepted as a positive reaction. The mean age was 10.2 ± 2.2 years (7.5-13 years) in Group I and 10.1 ± 2.1 (8-14) years in Group

II. Groups were comparable for age and gender ($p > 0.05$). The patients in Group I received subcutaneous standardized *Alternaria* extract (ALK®, 402, Denmark). All patients continued their concomitant medical therapies during the study period. Informed consent was obtained from parents. The study protocol was approved by the local ethics committee. In addition, written informed consent was obtained from each patient's family.

Daily peak expiratory flow (PEF) variability, monthly asthma symptom scores (ASS) and asthma medication scores (AMS), and pulmonary function tests (PFTs) were evaluated in all patients. Asthma quality of life questionnaire (AQLQ) scores, visual analog score (VAS), laboratory parameters (total IgE, *Alternaria*-specific IgE level), skin prick test, and methacholine and *Alternaria*-specific bronchial provocation tests were evaluated at the beginning and one year after SIT. In addition, blood and induced sputum eosinophil count and eosinophil cationic protein (ECP) levels were studied (Fig. 1).

Symptom and Medication Scores

Patients were taught, on their first day of treatment, how to mark their symptoms onto a standardized chart and to indicate which medication they had to take to counteract those symptoms. These charts reported the respiratory symptoms, as well as the possible necessary medications, for each day. The final AMS and ASS were calculated as the mean of the daily scores^{2,9}.

AQLQ and VAS

The AQLQ was performed at the beginning and the end of the study, according to previous reports by Juniper et al.^{10,11}. The severity of asthma symptoms were evaluated using a 100-mm VAS.

Challenge Tests

A methacholine challenge was performed to quantify non-specific bronchial hyperreactivity (BHR) before and after the study period. The challenge was carried out according to American Thoracic Society (ATS) guidelines¹² using a five-breath dosimeter (Koko Dosimeter, Ferraris Respiratory, Louisville, KY, USA) with

purified methacholine. Following a control inhalation of the diluent, each patient took 5 slow inhalations from functional residual capacity to total lung capacity from the starting concentration of 0.25 mg/ml. If the reduction in forced expiratory volume in 1 second (FEV1) was <20% from baseline, inhalations of increasing concentrations of methacholine, at 0.25 mg/ml, 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, and 16 mg/ml, were administered. Testing was continued until a 20% drop in FEV1 was obtained or the highest concentration was given.

Bronchial allergen provocation tests were performed 10 days after methacholine challenge using a dosimeter with controlled tidal breathing¹³. Allergen bronchial provocation tests were performed outside the *Alternaria* seasons, always at the same time of the year, in all patients at the beginning and at the end of the study. After baseline measurement of FEV1, the subject inhaled, from tidal volume, a saline solution and then, every 15 minutes thereafter, an *Alternaria* allergen solution. The solution contained a progressively increasing allergen dilution (1000, 3000, 10,000, 30,000, 100,000 SQ/ml). The challenge stopped when the FEV1 dropped by 20% (provocative dose for a 20% fall in FEV1 [PD₂₀]) or more, or when the full dose was administered without such a drop. At the end of the test, subjects inhaled β_2 -agonists as necessary (Fig. 1).

Sputum Induction, Processing and Analysis

Sputum was induced by inhalation of a hypertonic saline (4.5%) for 1, 2, 3, 4, 8 and 16 minutes (min) via an ultrasonic nebulizer (Hikoneb 906S, Kare Medical, Ankara, Turkey) as previously reported¹⁴. FEV1 was measured 1 min after each inhalation period. Subjects were asked to rinse their mouth and blow their nose, and try to cough between each dose of nebulized saline. Induction was continued until adequate sputum sample was obtained and stopped when the final inhalation (16 min) was given or if the patient developed either a decrease in FEV1 >20% or shortness of breath. As described in previous reports¹⁴, selected portions of the sputum were chosen by using an inverted microscope, and were treated with four volumes of Dithiothreitol (DTT; Sputalysin; Calbiochem Corp., San Diego,

CA, USA), which was diluted freshly in a 1:10 dilution with distilled water. The DTT-sputum mixture was homogenized in a shaking water bath at 37 °C for 20 min. An additional four volumes of phosphate-buffered saline (PBS) were added, followed by filtration through 50 ml filter and final centrifugation (790 g for 5 min). The supernatants were frozen at -80 °C for late fluid phase measurements. Cytospin preparations (Cytospin 4; Shandon Corp.) were made at 220 g for 6 min and stained with May-Grunwald-Giemsa for an overall differential cell count of 400 nonsquamous cells¹⁵. The results are expressed as percentage of total nonsquamous cells. Percentage counts of macrophages, eosinophils, neutrophils, epithelial cells, and lymphocytes were made over a total count of 400 cells. The same person, who was blinded to the clinical conditions of the patients, performed this evaluation.

Serum and Sputum ECP Assay

The concentrations of ECP in serum and sputum supernatant were measured with a fluoroenzyme immunosorbent assay (ImmunoCAP, Pharmacia; Uppsala, Sweden). The limit of detection of the fluid-phase assays was 2 μ g/L for ECP.

Measurement of Atmospheric *Alternaria* Spore Counts

The outdoor samples of airborne fungi were collected by Burkard 7-day recording volumetric trap in Adana atmosphere. The device was placed on the roof of the town hall at a height of 15 meters above the ground and samples were collected from November 2006 to October 2007. The air was sucked at a flow rate of 10 liters per minute and spore grains impacted on to tapes that were coated with a thin film of Vaseline-paraffin wax in toluene. The tape was then mounted with glycerine jelly, and 24 transverse travels were observed for a daily slide. Spores were identified visually with a light microscope at a magnification of 400x by an experienced aerobiologist. Fungal spore counts were expressed as fungal spores per m³ of air¹⁶.

Statistical Analysis

All of the analyses were performed using computer software (SPSS version 15.0; SPSS;

Chicago, IL, USA). Data are presented as the median (interquartile range), with the minimum to maximum range. The Wilcoxon signed-rank test was used for intragroup comparison and Mann-Whitney U test was used for intergroup comparison. Correlations were evaluated with the Spearman correlation test. Pre/post bronchial challenge tests comparisons and correlations were performed after log transformation of log normally distributed values. Pearson correlation test was used to test the relation between outdoor *Alternaria* spore counts and symptom-medication scores. A P value of less than 0.05 was considered significant.

Results

Alternaria-Specific IgE Levels

There was no difference in baseline *Alternaria*-specific IgE levels between groups ($p=0.09$); however, a statistically significant reduction was observed in Group I relative to Group II after one year ($p=0.0001$) (Table I).

Symptom and Medication Scores, VAS and AQLQ

When the groups were compared in terms of VAS, AMS and AQLQ, a statistically significant difference was found in the beginning of the study ($p=0.02$, $p=0.03$ and $p=0.01$, respectively). VAS was improved in both groups after one year. However, improvement in this parameter was more prominent in the SIT group. AMS, ASS and AQLQ did not change in either group at the end of the first year. When groups were compared for rates of within-group variations with respect to AMS, ASS and AQLQ between T0 and T1, a statistically meaningful difference was observed in Group I (Table I).

PFTs and Daily PEF Variability

There were no statistically significant differences in terms of PFTs and daily PEF variability between the groups at the end of the first year ($p>0.05$) (Table I).

Provocation Tests

In Group I, the mean PC_{20} value for methacholine bronchial provocation test (MBPT) was 0.82

± 0.94 (mg/ml) at baseline and 4.39 ± 3.36 (mg/ml) after one year ($p=0.002$), while PC_{20} values for MBPT did not change in Group II (Table I, Fig. 2). After the first year of treatment, a significant increase in the PD_{20} for *Alternaria* was found in Group I, and a 4-fold higher concentration was tolerated ($p=0.008$) (Table I, Fig. 2). However, there were no changes with respect to PD_{20} values in *Alternaria*-specific bronchial provocation tests in Group II ($p>0.05$). Similarly, no statistically significant difference was observed in the beginning of the study between the two groups in terms of MBPT PD_{20} , allergen bronchial provocation test PD_{20} , FEV1, PEF, and PEF variability ($p=0.1$, $p=0.3$, $p=0.4$, $p=0.3$, and $p=0.2$, respectively).

Changes in Eosinophil Counts and ECP levels

Although changes in blood eosinophil count following allergen provocation decreased after one year in the SIT group, this decline was not statistically significant ($p=0.066$). However, a rise in sputum eosinophil count following

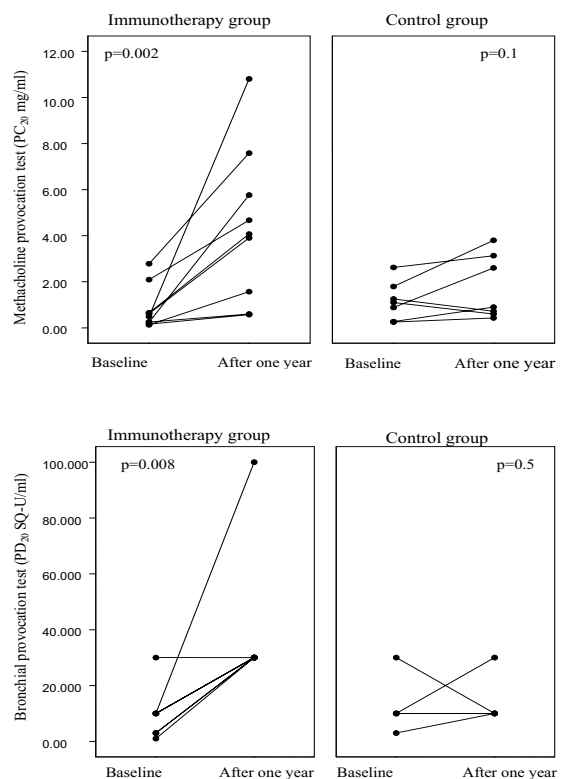


Fig. 2. Changes in the allergen bronchial provocation test and methacholine provocation test results.

Table 1. The Effect of *Alternaria* Immunotherapy on the Investigated and Laboratory Parameters

	Immunotherapy group (n=9)		P value (within Group I) T0-T1	Control group (n=7)		P value (within Group II) T0-T1	P value (between groups)
	T0 median (interquartile range) (min-max)	T1		T0 median (interquartile range) (min-max)	T1		
Visual analog scale	2.5 (2) (1.5-4.5)	7.5 (1.5) (5-9)	0.002	4.5 (1.5) (2.5-6)	6 (2) (4-9)	0.05	0.02 (T0) 0.03 (T1)
Quality of life score for asthma	3.8 (1.3) (2.73-5.21)	6.52 (0.57) (5.78-7)	0.002	4.91 (1.13) (3.91-5.82)	5.86 (2) (4.21-7)	0.01	0.01 (T0) 0.09 (T1)
Asthma medication score	2.51 (1.83) (1.51-4.61)	2 (1.5) (1-3.43)	0.01	1.38 (1.32) (0.48-3.64)	1.35 (1.10) (1.22-3.13)	0.4	0.03 (T0) 0.2 (T1)
Asthma symptom score	2 (1.38) (1-3.8)	0.54 (0.45) (0.12-1.26)	0.008	1.45 (0.39) (0.74-2.41)	0.76 (0.63) (0.5-1.46)	0.02	0.1 (T0) 0.08 (T1)
FEV ₁ (%)	73 (9.5) (60-80)	96 (19.5) (83-119)	0.008	75 (8) (65-97)	85 (15) (80-117)	0.02	0.4 (T0) 0.09 (T1)
FVC (%)	68 (8) (53-77)	89 (7) (79-100)	0.008	74 (7) (63-80)	89 (4) (79-94)	0.01	0.04 (T0) 0.4 (T1)
PEF (%)	76 (14) (64-91)	96 (13.5) (81-102)	0.007	74 (17) (57-93)	101 (13) (73-106)	0.02	0.3 (T0) 0.2 (T1)
PEF variability (%)	12.6 (7) (7.24-25.01)	4.03 (5.01) (3.36-9.75)	0.008	11.5 (5.35) (7.98-15.65)	8.13 (2.74) (5.28-11.34)	0.01	0.2 (T0) 0.09 (T1)
MBPT PD ₂₀ (mg/ml)	0.49 (1.17) (0.13-2.78)	4.07 (5.59) (0.57-10.8)	0.002	1.10 (1.52) (0.25-2.62)	0.90 (2.53) (0.43-3.80)	0.1	0.1 (T0) 0.03 (T1)
Bronchial provocation test PD ₂₀ (SQ-U/ml)	10000 (7000) (1000-30,000)	30000 (0) (30,000-100,000)	0.008	10000 (13500) (3000-30,000)	10000 (10000) (10,000-30,000)	0.5	0.3 (T0) 0.006 (T1)
<i>Alternaria</i> -specific IgE (IU/ml)	26.4 (21.8) (7.81-52.1)	8.17 (14.2) (2.63-22.8)	0.004	35.3 (19) (19.3-54.7)	46.8 (28.4) (17-92.6)	0.05	0.09 (T0) 0.0001 (T1)
Total IgE levels (IU/ml)	185 (251.3) (64.9-1937)	130.3 (144.6) (19.8-325)	0.05	125 (159) (65-628)	190.1 (191.2) (93.6-428)	0.3	0.4 (T0) 0.06 (T1)

FEV₁: Forced expiratory volume in 1 second. FVC: Forced vital capacity. PEF: Peak expiratory flow. MBPT: Methacholine bronchial provocation test

T0: The results of clinical and laboratory parameters at the beginning of the study

T1: The results of clinical and laboratory parameters after 12 months of study

allergen provocation decreased significantly ($p=0.011$). We did not demonstrate any differences regarding these parameters in Group II ($p=0.932$, $p=0.866$; respectively). Serum ECP levels before allergen provocation did not change in Group I ($p=0.767$), while it increased in Group II ($p=0.043$) at the end of the first year. However, serum ECP levels after allergen provocation were found to be higher in both groups ($p=0.011$, $p=0.043$). There was no statistically significant change in sputum ECP levels in Group I before and after allergen challenge ($p=0.138$, $p=1.000$), whereas sputum ECP levels were found to be elevated after allergen challenge in Group II ($p=0.028$) (Table II).

Atmospheric Spore Count and Symptom Scores

The outdoor *Alternaria* spore concentrations were significantly correlated with the monthly ASS in Group I ($r= -0.565$, $p=0.05$), while they were not significantly correlated in Group II ($r= -0.377$, $p=0.2$). However, the outdoor *Alternaria* spore concentrations and the mean monthly AMS were not significantly correlated in either group (Group I, $r= -0.384$, $p=0.2$; Group II, $r= -0.305$, $p=0.3$). The main peak for *Alternaria* spore was detected between May and August, and ASS were observed to be lower in Group I compared to Group II during this period, which corresponded to 9-11 months of immunotherapy. However, a statistically significant difference between groups was only observed for ASS in August ($p<0.05$) (Fig. 3).

Discussion

In contrast to other allergens, relatively few studies have investigated the role and efficacy of specific IgE-mediated immunotherapy with fungal extracts in subjects with respiratory allergies. The reason for this is probably related to the current inadequate standardization of skin tests for fungal antigens and to the different antigenic potential of fungal species. The efficacy of SIT has been demonstrated in controlled studies using extracts of *Alternaria* and *Cladosporium*. These studies indicated that SIT with fungal extracts can be used in well-selected monosensitized patients with compliant anamneses and provocation

test results²⁻⁵. Similar to these studies, we have demonstrated that subcutaneous immunotherapy with *Alternaria* affected clinical and laboratory parameters in asthmatic children at the end of the first year.

The VAS is a reliable test that allows evaluation of the improvement in health status and quality of life of the patient and demonstrates a positive correlation between clinical improvement and laboratory parameters². Pajno et al.¹⁷ reported that improvement in VAS became apparent following 16 months of SIT. Consistent with these reports in our study, VAS improved in the SIT group at the end of the first year of the study.

In patients with asthma and/or rhinitis in *Alternaria* SIT groups, improvement in symptoms and medication scores has been observed previously at the end of the first year, with substantial improvement detected after three years of immunotherapy^{2,3}. In our study, no statistically significant differences were detected in ASS and AMS between groups after one year. On the other hand, ASS and AMS were found to be lower in the SIT group during the *Alternaria* season, which suggests that immunotherapy was effective. When the groups were compared in terms of VAS, AMS and AQLQ, a statistically significant difference was found in the beginning of the study. Evaluation of these parameters is highly dependent on the patient's compliance. We think the individual differences and small number of the patients were effective on the emergence of these differences in the beginning of the study. However, no difference was found in the beginning of the study in respiratory parameters such as FEV1, PEF and PEF

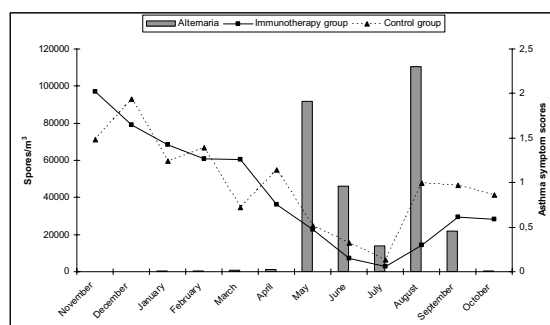


Fig 3. The relationship between *Alternaria* spore concentrations in the atmosphere with asthma symptom scores in both groups.

Table II. Pre- and Post-Challenge Results of Laboratory Parameters of All Subjects During the Study Phases

	Immunotherapy group (n=9) median (interquartile range) (min-max)				P value (within group)	Control group (n=7) median (interquartile range) (min-max)				P value (within group)	P value (between groups)
	BP0	AP0	BP1	AP1		BP0	AP0	BP1	AP1		
Blood eosinophils (mm3)	510 (347.5) (205-893)	640 (867.5) (410-2100)	440 (445) (150-840)	620 (460) (280-1350)	*P=0.004 **P=0.002 ***P=0.155 ****P=0.066	440 (180) (100-800)	920 (620) (440-1620)	550 (480) (170-760)	700 (850) (330-1850)	*P= 0.008 **P=0.008 ***P=0.735 ****P=0.932	0.3 (BP0), 0.3(AP0) 0.4 (BP1), 0.3(AP1)
Serum ECP levels (mcg/L)	15.8 (14.2) (3.7-42.3)	22.9 (23) (5.4-36)	18.4 (19.1) (11.4-72.8)	40.3 (52.1) (8.9-96.5)	*P=0.4 **P=0.02 ***P=0.767 ****P=0.011	17.7 (18.4) (4.9-49.6)	26.3 (43.6) (6.3-68)	33.4 (47.2) (4.9-70.9)	55.9 (46.2) (14.4-76.3)	*P=0.07 **P=0.05 ***P=0.043 ****P=0.043	0.4 (BP0), 0.2(AP0) 0.1 (BP1), 0.4(BP1)
Sputum ECP levels (mcg/L)	0 (36) (0-79.5)	235.2 (481.6) (0-684)	0 (133.2) (0-445.2)	36.8 (532.8) (0-2400)	*P=0.008 **P=0.04 ***P=0.138 ****P=1.00	23.4 (101.4) (0-240)	114 (222) (0-438)	339.6 (847.5) (0-1035)	995.4 (1192.5) (65-1354)	*P=0.03 **P=0.01 ***P=0.068 ****P=0.028	0.2 (BP0), 0.2 (AP0) 0.1 (BP1), 0.03 (AP1)
Sputum eosinophil counts (%)	7.8 (8.65) (2-17.7)	17.9 (10.07) (5.2-35)	4.9 (6) (1.9-12.7)	10.3 (6.6) (5.8-15.3)	*P=0.004 **P=0.006 ***P=0.139 ****P=0.011	8.1 (7.1) (2.4-12.2)	10.3 (8.7) (6.9-50.2)	9.75 (6) (4.2-12.4)	17.7 (4.5) (2.2-21.7)	*P=0.04 **P=0.01 ***P=0.127 ****P=0.866	0.4 (BP0), 0.1 (AP0) 0.1 (BP1), 0.01 (AP1)

ECP: Eosinophilic cationic protein.

* = BP0 vs AP0

** = BP1 vs AP1

***: BPO vs BP1

****: AP0 vs AP1

Difference in changes after allergen provocation at the end of the first year.

BP0: The results before the start of allergen bronchial provocation test at the beginning of the study.

BP1: The results 17 h after the start of allergen bronchial provocation test at the beginning of the study.

AP0: The results before the start of allergen bronchial provocation test after 12 months of study.

AP1: The results 17 h after the start of allergen bronchial provocation test after 12 months of study.

variability, indicating severity of the disease and airway obstruction, and bronchial provocation tests, showing direct organ response. Evaluation of ASS and AMS in patients with asthma and rhinitis is considered to be important for clinical parameters in the follow-up of these types of diseases². When our study population was evaluated regarding monthly AMS, ASS and concentration of *Alternaria* spores in the atmosphere, ASS demonstrated a statistically significant correlation in Group I, while it did not show any correlation in Group II. ASS were observed to be lower in Group I compared to Group II during this period, which corresponded to 9-11 months of SIT.

This study demonstrated that SIT with *Alternaria* extracts appeared to improve indices of allergic inflammation determined by BHR and eosinophil counts in sputum. SIT is known to reduce both immediate as well as late-phase allergen-induced symptoms, by acting both on humoral as well as on cellular immune mechanisms involved in allergic inflammation. In a number of studies, SIT was shown to inhibit both the recruitment and activation in mucosa of proinflammatory cells involved in the allergic reaction. Successful SIT has been associated with a decrease in the recruitment of mast cells, basophils and eosinophils in the skin, nose, eye, and bronchial mucosa, following provocation or natural exposure to allergens^{18,19}.

In patients who received *Alternaria* SIT, a decrease in specific and non-specific bronchial reactivities was detected²⁰. In our study, while no statistically significant difference between groups in response to *Alternaria* and MBPTs was detected at the beginning, a significant increase in test concentrations in Group I was seen after one year, which was interpreted as an indication of suppression of allergic inflammation under the influence of immunotherapy. We did not observe these effects in Group II. Eosinophilia and ECP have been considered to have an important role in the pathogenesis of allergic diseases. Serum levels of ECP have thus been considered useful to monitor airway inflammation in asthma as it correlates with sputum eosinophil counts. SIT has been shown to decrease serum ECP²¹. In our study, consistent with other studies²², statistically significant decreases in eosinophil

counts and sputum ECP after allergen challenge in Group I relative to Group II at the end of the first year were considered to be associated with a delayed phase inflammatory response subsequent to encounter with allergen, as a result of immunotherapy. Serum ECP level was shown to decrease after two years but not after one year of mite SIT in Chinese perennial allergic rhinitis²³. However, the rise in serum ECP after allergen challenge was significantly attenuated after just one year of SIT in asthmatic patients with *Alternaria* allergy.

With respect to allergen-specific antibody responses, SIT often induces an initial increase in specific IgE levels and subsequent downregulation in the following months. Moreover, successful SIT protocols resulting in clinical improvement of patients often elicit allergen-specific IgG responses (mostly IgG1 and IgG4), and in a few reported cases, IgA responses²⁴. However, in some immunotherapeutic studies performed with fungal extracts, a statistically significant decrease in specific IgE levels was observed at the end of the first year^{5,25}. Similarly, our results also demonstrate that specific IgE levels at the end of one year in the SIT group decreased when compared with Group II. This has been interpreted as showing that the concentrations of fungal extracts used have been adjusted properly to prevent the induction of sensitization.

Despite reports indicating that SIT does not substantially influence PFTs⁷, some studies have reported improvements in response to SIT at the end of a one-year therapy, at a rate of 16% in FEV1, and 4.5% in other PFTs and PEF meter measurements^{17,26}. In our study, no statistically significant differences were detected with respect to PFTs between groups after one year. However, in the SIT group, despite lower baseline FVC and FEV1 values relative to Group II, these parameters reached higher levels in the SIT group than in Group II at the end of the first year. In addition, both groups showed a small but not statistically significant difference in daily PEF variability, which could be explained by improved compliance associated with study participation. Therefore, one year of treatment, as in our case, is probably not sufficient to demonstrate a difference. A

decrease in specific and non-specific bronchial reactivities has been detected in patients who received *Alternaria* SIT²⁰. We did not find any statistically significant difference between groups in response to *Alternaria* and MBPTs at the beginning, while there was a significant increase in test concentrations in Group I after one year.

In our study, since an inclusion criterion was the presence of an isolated hypersensitivity to *Alternaria*, and also since eight patients discontinued the therapy within the first year of the study, only a small number of cases completed the present study. This limited number of patients might have affected the power of the statistical tests that were performed. In addition, the smaller number of patients and evaluation of SIT within a relatively short time period of one year might suggest unfavorable or very feeble effects for SIT. Although we observed significant improvement in immunological parameters in the immunotherapy group, there was no statistically significant difference between the two groups with respect to clinical parameters. Some authors have pointed out that the clinical documentation of the efficacy of SIT is affected by the duration of the treatment, by the amount of allergen administered, and by the criteria for patient selection²⁷.

In conclusion, significant responses were obtained in immunological parameters and end-organ response such as bronchial challenge tests, *Alternaria*-specific IgE levels and sputum eosinophil count and ECP levels even at the end of the first year of this five-year study. In addition, some clinical parameters including VAS improved in the SIT group. Evaluation of the effects of all parameters on SIT at the end of the five years will be more enlightening. In light of all these findings, we suggest that long-term studies should be made for the clinical documentation of the efficacy of SIT with *Alternaria* in allergic children.

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