Evaluation of New Sensitizations in Asthmatic Children Monosensitized to House Dust Mite by Specific Immunotherapy

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SUMMARY Specific immunotherapy (SIT) is one of the treatment modalities recomended for the management of asthma and allergic rhinitis by international guidelines. A potential benefit of immunotherapy (IT) is to prevent the development of sensitisation to new allergens. There is stil no conclusion on this subject. One hundred twenty-two children 8-18 years old with intermittent asthma, with or without allergic rhinitis, all of whom were monosensitised to house dust mite (HDM) were selected. Sixty two of these children accepted to receive SIT with HDM extract for 4 years and the remaining 60 did not accept SIT and were treated with asthma medications only. This second group of children served as the control group. At the end of the 4-year study period, 36 of the 53 patients (67.9%) in the SIT group showed no new sensitizations, compared to 38 of 52 (73.0%) in the control group (p = 0.141). The most frequent new sensitizations at the end of the study were pollens, grasses and olive polen, followed by animal dander, alternaria and cockroach. In conclusion, SIT may not prevent the onset of new sensitizations in asthmatic children monosensitized to house dust mites. Further investigation is required to clarify the immunologic mechanisms and other factors by which SIT reduces or not the development of new sensitizations in monosensitized children.

Specific immunotherapy (SIT) is a technique by which increasing amounts of allergen extract are injected subcutaneously over a varying number of months to years to lessen clinical symptoms that arise on exposure to allergens. The efficacy of immunotherapy with allergen extracts has been demonstrated by the early work of Noon[1] and Freeman[2] and now one of the treatment modalities recommended for the management of asthma and allergic rhinitis by international guidelines.[3] Clinical efficacy of SIT in allergic asthma and/or rhinitis has been confirmed by controlled studies[4-6] and recent metaanalysis including latest clinical trials.[7,8] These studies conclude that SIT significantly reduces asthma symptoms and use of asthma medication and bronchial hyperreactivity. The effectiveness is revealed to last for 3-4 years after its discontinuation.

Another potential benefit of SIT is to prevent the development of sensitization to new allergens.

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The hypothesis that SIT could prevent the development of new allergen sensitizations especially in mono-sensitized patients was first reported by Des Roches *et al.*[9] This theory was then confirmed by other investigations.[10-12] Recent years increasing evidences coming from the controlled studies suggest and as it is now widely accepted that SIT did not prevent the development of new allergen sensitizations in mono-sensitized patients.[13-15]

Thus, this study aimed to investigate the effect of SIT on the development of new sensitizations in asthmatic children monosensitized to house dust mite (HDM). In this study asthmatic children receiving SIT were compared with a control group treated pharmacologically for the development of new sensitizations. The aim was to determine whether IT in monosensitized patients to HDM prevented new sensitizations to common allergens present in the locality.

MATERIAL AND METHODS

Study population

This open label prospective clinical trial performed between years 2004 to 2008 in the Outpatient Clinic of Pediatric Asthma and Allergy Department in Ankara Dışkapı Children Health Education and Training Hospital. One hundred twenty two children with asthma between the ages of 8 and 18 years who have monosensitized to HDM were selected. The inclusion criteria included: a) age between 8 and 18 years; b) a clinical history of allergic rhinitis and/or mild-to-moderate asthma as defined by the Global Initiative for Asthma (GINA) report,[3] with symptoms lasting at least 1 year, with or without allergic rhinitis; and c) monosensitization to house dust mite (*Dermatophagoides pteronyssinus* and/or *D. farinae*). The exclusion criteria included: a) children with severe asthma; b) children having concomitant sensitization to inhalant allergens other than HDM such as cockroach, mold species, cat and dog dander and pollens; c) children receiving previous SIT; and d) children having contraindications for SIT. The study was approved by the ethics committee of Dışkapı Children Health Education and Training Hospital. The children's parents were asked to give their informed consent in writing.

Skin prick tests (SPT)

Skin prick tests were performed on the volar surface of the forearm according to European Academy of Allergology and Clinical Immunology (EAACI) recommendations[16] as follows: the SPT was performed with a panel of 10 most common aeroallergens (Stallergenes, France) by the use of standard prick method. All skin prick tests were done by specialists in pediatric allergy in our clinic and the results were evaluated by the same physicians. The standard respiratory allergen panel included tree pollens, grass pollens, dust mite, fungus, animal dander, grains pollen, wild grass, flower pollens and latex. Histamine hydrogen chloride 10 mg/ml was used as the positive control and physiologic saline as the negative one. A wheal diameter of at least 3 mm greater than those of the negative controls (15 minutes after administration) were considered positive if no dermographism and/or positivity of the negative control was recorded. All patients were instructed not to take medications during the 2 weeks before the test.

Experimental groups

SIT was recommended to all patients after diagnosis. Sixty-two patients who accepted receiving SIT with HDM extract for 4 years submitted into the SIT group. Sixty patients who did not accept SIT (mainly because of its cost, inconvenience, or travel difficulties) included into the pharmacotherapy group and treated only with madication for at least 4 years and were not given the placebo injections following the same schedule as the SIT group. This second group of children included as the control group.

Immunotherapy protocol

Biologically standardized depot preparations of mite mix (*D. pteronyssinus* and *D. farinae*) were used for 4 years in the SIT group. The preparation was calcium phosphate adsorbed extracts obtained from pure mite culture (APSI Retard, Stallergenes, Antony, France). The Stallergenes preparations were supplied as having indices of reactivity (IR) of 0.01, 0.1, 1 and 10. The induction phase was performed according to the manufacturer’s recommendations and was followed by a perennial schedule with

maintenance injections (0.8 ml, the maximum individual dose tolerated by all patients at 4-week intervals). Specifically, injections were postponed if other diseases were present and the dose was lowered to the preceding dose at the next visit if a local reaction greater than 3 cm in diameter appeared or it was halved if a systemic reaction occurred. Patients were kept under observation for 30 minutes after each administration. The interval between injections was 6 ± 2 weeks.

Pharmacological treatment

Patients in both groups were allowed to use medication throughout the study according to GINA recommendations for children suffering from intermittent asthma.[3] All patients, whether treated with SIT or not, were prescribed and instructed to use the same antiallergic drugs to control their respiratory symptoms. Antihistaminics, inhaled beta-2 adrenergic agonists, topical and systemic steroids and leukotriene antagonists were administered to the patients as needed.

Assessments

After the initial study to select the patients, each patient was submitted yearly to skin prick tests with the standard panel of allergens throughout the follow-up period. An allergen specific IgE test could not be performed because of technical insufficiency of our laboratory.

Side-effects of SIT early (within 1 hour) side-effects of each allergen administration were recorded at the clinic. The children's parents were also instructed to observe and report any delayed side-effect (swelling at the injection site, rhinitis, asthma, urticaria, angioedema, etc.) to the clinic.

Statistical analysis

SPSS for Windows 11.5 package programme was used for statistical analysis. Normality of the distributions was evaluated with the Shapiro-Wilk test. Groups were compared by using student's *t* test for normally distributed continuous variables, and by the Mann Whitney U test for variables with non-homogenous distributions. Nominal variables were analyzed with Pearson chi square test. All *p* values were considered statistically significant if $p < 0.05$.

RESULTS

One hundred twenty two asthmatic patients with a single sensitization to house dust mite allergen were included in this study. A total of 105 children (86.7%) completed the 4 years study period; 9 patients in the study group and 8 patients in the control group were excluded from the study. Groups were similar when compared according to the demographic characteristics as observed in Table 1. Boys were slightly higher in Pharmacotherapy (control) group compared to SIT group (46% *vs*. 34%, $p = 0.047$). Asthma severity was similar in both groups at the beginning of the trial and majority of patients were mild (intermittent or persistent) asthma in both groups ($p = 0.473$).

Adverse events related to immunotherapy

There were no life-threatening reactions during the trial.

Evolution of sensitization

At the end of the 4-year study period, 36 of the 53 patients (67.9%) in the SIT group showed no new sensitizations, compared to 38 of 52 (73.0%) in the control group ($p = 0.141$). The most frequent new sensitizations at the end of the study were pollens, grasses and olive pollen, followed by animal dander, alternaria and cockroach (Table 2). Twelve of 17 (70.6%) patients in the SIT group and 11 of 14 (78.6%) patients in the control group showed multiple (2 or more) new sensitizations at the end of the study ($p = 0.815$) (Table 3). Seven of 17 (41.1%) new sensitizations in SIT group and 6 of 14 (42.8%) in control group occurred at second year of the therapy, and the others occurred at 4 years of the therapy. The most frequent new sensitizations at the end of the study were to grass pollens. The next most frequent were to *Meadow fescue* (*Festuca elatior*) and *Oleceacea* pollens (Table 2).

DISCUSSION

In the present study, 53 children with asthma with or without rhinitis aged between 8 and 18 years, monosensitized to house dust mite, received SIT for 4 years with adsorbed extracts and were evaluated and compared with 52 children monosensitized to

house dust mite who received only pharmacologic treatment (control group). The comparison was based on the development of new sensitization. New sensitizations to inhalant allergens developed 32.1% in SIT group and 27% in control group ($p = 0.141$). This finding of our study showed that SIT did not prevent new sensitization to inhalant allergenes.

Although some studies showed that SIT significantly prevent new sensitization in both adults and children,[9-11,17,18] it is now widely accepted that SIT did not prevent the development of new allergen sensitizations in monosensitized patients.[13-15] Asero[13] compared 284 adult patients receiving immunotherapy with standardized tree pollen extracts with a parallel group of 407 patients treated pharmacologically. After the period of 2 years, surprisingly, the prevalence of new sensitizations was significantly higher among subjects receiving SIT (132/284, 46%) than among those not receiving SIT (95/407, 23%; $p < 0.001$). Twenty-seven subjects developed new sensitization to birch and/or ragweed pollen while undergoing SIT. These findings were consistent in all subgroups with different airborne allergies.[13] In a more recent study, the same author studied asthmatic groups receiving SIT or pharmacotherapy for having polysensitizations (five or more new sensitizations) did not reveal a difference.[14] The prevention of new sensitizations in asthmatic children monosensitized to house dust mites by specific immunotherapy has been evaluated in another recent study conducted in our country by Gulen *et al.*[15] In this study, 129 children aged from 6 to 10 years were divided in the immunotherapy and control groups who were treated only with symptomatic medication. Immunotherapy was given for 4 years and the follow up was 6 years. At the end of the study, the prevalence of new sensitizations was significantly higher in the SIT group (45.5%) than in the control group (18.1 %).

The mechanisms that explain the effect of new sensitizations in children given immunotherapy are unclear. One of the most intriguing field of research centers on the possible action of immunotherapy on the regulation of the Th1:Th2-balance, lymphocyte function and responsiveness to allergen, and the production of cytokines and interferon-gamma (IFN-gamma). Immunotherapy has been shown to alter the production of cytokines and IFN-gamma, and in addition to that, to decrease the number of mast cells in the skin. Clinical improvement seen after allergen immunotherapy for allergic diseases such as

Table 1 Baseline characteristics of the study population

	SIT group (n = 53)	**Control group** (n = 52)	***p***
Age*	12.7 ± 2.4	12.0 ± 2.4	0.134[a]
Gender (male), n (%)	34 (65.4)	23 (46.0)	0.049[b]
Family history of asthma, n (%)	18 (34.6)	16 (32)	0.835[b]
Asthma and rhinitis	24 (46.2)	21 (42)	0.695[b]
Asthma severity			
Mild, n (%)	39 (75)	41 (82)	0.473
Moderate, n (%)	13 (25)	9 (18)	
FEV1*	91.2 ± 21.3	90.6 ± 14.1	0.452[c]
FVC*	95.9 ± 17.1	96.2 ± 13.7	0.415[c]
FEV1/FVC*	87.4 ± 5.5	85.4 ± 6.6	0.245[c]
FEF 25-75*	95.4 ± 25.2	94.3 ± 23.2	0.548[a]
PEF*	83.6 ± 17.1	85.2 ± 15.4	0.258[a]

*Data are mean ± SD.
a, Student's *t* test; b, Chi-square (χ^2) tests; c, Mann Whitney U test;

rhinitis and asthma is associated with the induction of IL-10 and TGF-beta producing Tr-1 cells as well as Foxp3 expressing IL-10 T cells, with resulting suppression of the Th2 cytokine milieu.[19-24] But these effects are somewhat allergen specific and the effect of SIT on the sensitization to new allergens is not well understood yet. Furthermore, it has been suggested that the function of regulatory T cells which are supposed to play a major role in the effect of SIT is under genetic control and an alteration in the function of these cells can present different clinical pictures.[15] For this reason, individual or regional variations may be seen as a result of SIT administration. There are three investigations conducted in different parts of our country (Aegean, Mediterranean and central Anatolia). Our study and the report by Gulen *et al.*[15] did not find a preventive effect of SIT for new sensitizations whereas Inal *et al.*[12] reported the contrary. There might be some factors accounting for the failure of the current study to reduce future allergic sensitizations and effect a statistically significant difference between the two groups; 1) allocation to SIT and control groups were on the basis of patient choice (*i.e.* the study was not randomized and placebo-controlled) probably influenced the results. In fact, although patients receiving and not receiving SIT did not show any baseline difference in age, or severity of asthma, during the follow-up, severity of asthma and drug use in SIT group were significantly decreased than

Table 2 Distribution of new sensitizations developed in the study groups*

Patients	SIT group	Control group	*p*
Number of patients enrolled	62	60	
Patients followed-up for 4 years	53 (85.4)	52 (86.6)	
New sensitizations			
None	36 (67.9)	38 (73)	0.141
Grass	12 (70.6)	11 (78.6)	0.698
Meadow fescue (*Festuca elatior*)	7 (41.2)	8 (57.1)	0.479
Oleceacea	6 (35.3)	4 (28.6)	0.690
Cat	3 (17.6)	4 (28.6)	0.671
Dog	2 (11.8)	2 (14.3)	0.835
***Alternaria* spp.**	2 (11.8)	3 (21.4)	0.626
Cockroach	1 (5.9)	2 (14.3)	0.431

*Data are presented as n (%).

Table 3 Categorical distribution of new sensitizations*

	SIT group	Control group	*p*
1 new sensitization	8 (47.1)	6 (42.9)	0.906
2 new sensitizations	3 (17.6)	2 (14.3)	
3 or more new sensitization	6 (35.3)	6 (42.9)	
Monosensitization	8 (47.1)	6 (42.9)	0.815
Polysensitizations (2 or more)	9 (52.9)	8 (57.1)	

*Data are presented as n (%).

control group (data are present in our other article which is under review); 2) we used biologically standardized depot preparations of allergen extracts of HDM used in this study which obtained from pure mite culture (APSI Retard, Stallergenes, Antony, France) and doses of SIT were given to the patients according to manufacturer's recommendations. Therefore, we do not think that quality of extract or dose of SIT effect our results; 3) the number of boys were slightly high in SIT group, but there is no data untill today that boys are more suceptible than girls in the meaning of development of new sensitization during SIT; 4) we exclude patients who had pollen allergy at the enrollement. As there are more cross-reactions between the pollens, to avoid expected misdiagnosis of new sensitizations we choose only patients who have monosentized to HDM.

Certain longitudinal studies have reported an increase in the sensitization rate from childhood to adulthood.[25,26] One of them was carried out on children and concluded that the evolution from mono- to polysensitization was age-related.[26] In another study the same authors reported that the rate of development of polysensitization was 43.6 % in previously monosensitized children after 2 to 10 years from the first diagnosis.[27] They found that 45.4% of the patients who were monosensitized to house dust mite became polysensitized. Another study by Inal[12] who reported that new sensitization in SIT group were significantly lower than control group, they found slightly higher rate of polysensitization (53.3%) in their control group. We found a higher rate of polysensitization in our study (52.9% and 57.1% in SIT and control group, respectively). This means that polysensitization in our study in both groups is concordance with the literature.

In the present study, Grass pollens, *Meadow fescue* (*Festuca elatior*) and olive tree were the most common allergens responsible for the new sensitizations. This finding is not surprising, considering that in Central Anatolian Region, these are common pollens and the pollen season is generally very long with high pollen counts. The pollen season lasts from late February to early November.[28,29] Since sensitization to pollen allergens generally occurs at older ages than sensitization to house dust mite,[30,31] it cannot be excluded that many of the children in our study carried a genetic predisposition to pollen allergy that was still clinically unexpressed when they were enrolled. An increased sensitization rate from childhood to adulthood has been shown by several longitudinal studies,[25,27,32] and this propensity seems particularly evident in children sensitized to house dust mites.[27] Moreover, when these subjects were enrolled in our study, may be they had not yet been exposed to these 'new' allergens long enough to become sensitized. Accordance with this we found that nearly 40% and 60% of new sensitizations occured at the second year and fourth years of the of the study. These findings partly show that although, SIT reduced symtoms and drug used, may not be able to alter the genetic predisposition to sensitization to allergens.

In conclusion, according to our data, SIT may not prevent the onset of new sensitizations in asthmatic children monosensitized to house dust mite. Further investigation is required to clarify the immunologic mechanisms by which SIT reduces or not the development of new sensitizations in monosensitized children.

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