

Allergen-Vaccine Immunotherapy And Its Effect On Immunological Markers In Asthmatic Children

¹Hanan A. Fathy, ²Gihan A. Fathy, ²Essam M. Galal, ³Nagwa Abd EL-Ghaffar and
⁴Amal Elmenshawy

¹Pediatric Department-National Center for Radiation Research and Technology.

²Department of Child Health, National Research Centre.

³Clinical and Chemical Pathology, National Research Center, Egypt.

⁴Pediatric Department, Research Institute of Ophthalmology.

Abstract: Allergen immunotherapy is the administration of gradually increasing quantities of an allergen vaccine to an allergic subject, reaching a dose which is effective in ameliorating the symptoms associated with subsequent exposure to the causative allergen. So allergy vaccine immunotherapy is a treatment that can modify allergic disease. In the present study we evaluated a period of one and half year of house dust mite immunotherapy on the concentrations of two immunologic markers: Eosinophil cationic protein (ECP) and nitric oxide (NO). We also compared the effect on asthma symptoms, allergen specific bronchial challenge test and the skin prick test. The immunotherapy was performed on 36 mite allergic, asthmatic children (age range from 6-15 years) were included in our study. Twenty of the cases were treated with sublingual immunotherapy (55.5%) and 17 cases were controls as they refused to receive the medication. Efficacy was evaluated clinically on asthma symptoms and by measuring the serum NO and ECP, allergen specific bronchial challenge test and the skin prick test. Results: The sublingual immunotherapy (SLIT) group detected a significant improvement in asthma symptoms ($P=0.001$) and skin reactivity to dermatophagoides pteronyssinus ($P=0.020$) whereas the control group did not. The result of bronchial challenge to D pteronyssinus showed a similar pattern at baseline and after 2 years of treatment in both groups. The serum levels of NO and ECP were significantly reduced in the SLIT group ($P=0.01$ and $P=0.018$) compared to baseline, whereas the values remained the same in the control group. The result of bronchial challenge to D pteronyssinus showed similar results at baseline after 2 years of treatment in both groups. The tolerated allergen concentration increased in both groups ($p<0.05$). Lung function tests, total immunoglobulin (IgE) and specific IgE to D pteronyssinus and Dermatophagoides farinae did not change after 2 years of treatment in either group. Conclusion: The SLIT with D pteronyssinus improves the clinical parameters and the immunological parameters in mite allergic asthmatic children after one and half year of treatment. The skin prick test may be used as a marker of efficacy of therapy.

Key words: Asthma-specific immune therapy-serum ECP and serum NO.

INTRODUCTION

Allergen-specific Immunotherapy which is also called allergen immunotherapy, hypo-sensitization therapy or desensitization, involves administration of increasing concentrations of antigen-specific extracts to allergic patients with the goal of inducing a state of immunologic tolerance for the purpose of reducing or eliminating the patient's adverse clinical response on subsequent natural exposure to these allergens with the aim to alleviate symptoms during exposure to the allergen. (*Durham, 2006*).

It is an FDA-approved, clinically effective method and induces long-term remission of allergic rhinitis and allergic asthma, with improvement in clinical symptoms. (*Passalacqua et al., 2007*).

Defining allergens as well as cells such as regulatory T-cells and characterizing the antibodies involved in the pathogenesis (including blocking antibodies) have helped very much towards a better understanding of the immunologic process. However, Allergen specific immunotherapy (SIT), as a specific curative treatment for allergy also dates back to the beginning of the previous century and has progressed considerably during these years. SIT similar to natural immunomodulation, directs the immune response towards tolerance. (*Bidak et al., 2011*).

Successful immunotherapy results not only in the increase of allergen concentration necessary to induce immediate or late-phase reactions, but also in the decreased responses to nonspecific stimulation. (*Nilson, 2007*).

Therefore, in contrast to symptomatic treatment, it can reduce the likelihood of developing additional sensitizations by interrupting the so-called "atopic march" and patients may benefit from persistence of alleviation of clinical symptoms. (*Senti et al., 2010*). So, allergen-specific immunotherapy, together with drug therapy and allergen avoidance, is a cornerstone in the management of respiratory allergy in children.

Crossponding Author: Hanan A. Fath, Pediatric department-National Center for Radiation Research and Technology.

In order to increase the safety of immunotherapy and provide an easier administration, non-injection routes such as nasal, oral, sublingual swallow and sublingual-spit techniques were developed. In nasal immunotherapy, an aqueous solution or powder in capsules (which are to be broken later), are sprayed into the nose through an appropriate device to avoid inhalation into the deep airways. Thus, by introduction of sublingual immunotherapy, the use of nasal immunotherapy is declining. (Canonica *et al.*, 2003).

In oral immunotherapy (SLIT), which is shown to be effective, economic saving, and relatively safe, antigens in aqueous solutions, tablets or gastro resistant capsules are swallowed (Green *et al.*, 2010). In sublingual swallow technique antigen is held for 1 to 2 minutes under the tongue and then is swallowed, while in sublingual spit technique the antigen is spit out after 1 to 2 minutes under the tongue. (Leatherman *et al.*, 2007). SLIT can be used co-seasonally, pre-seasonally or continuously. (Mohapatra *et al.*, 2010).

Many extracts are standardized either biologically or immunologically. The clinical efficacy of SCIT is now well established (Schau *et al.*, 2009) and regarding safety, though systemic reactions are not frequent, careful administration is recommended. (Rank *et al.*, 2008). In Sublingual immunotherapy, Extracts may be standardized either biologically or immunologically. It was well tolerated with local and self-limiting side effects. (Passalacqua *et al.*, 2007).

The differences in sublingual and subcutaneous methods may be due to differences between oral antigen presenting cells (APCs) and Langerhans cells and their skin counterparts. (Pajno, 2007).

However; similar to subcutaneous immunotherapy, sublingual method in allergic subjects causes a reduction in proliferative responses of T- cells, their cytokine production, the number of eosinophils and neutrophils, induction of systemic Treg cells, an increase in interleukin (IL)-10 production, and decreased bronchial reactivity to methacholine. It also causes antigen-specific IgE decrease, early rise in IgG1, late increases in IgG4. The majority of publications comparing active sublingual immunotherapy with placebo or controls showed efficacy of this method for rhinitis, conjunctivitis, and/or asthma. Contrary to subcutaneous immunotherapy that has a high potential for severe reactions, sublingual route has minimal risk. (schaub *et al.*, 2009 & Mohapatra *et al.*, 2010)

To date, no severe or life-threatening adverse events have been reported and the majority of events (asthma exacerbation, rhino-conjunctivitis, oral cavity pruritis, throat irritation, rhinitis, itchy eyes, nausea, and GI complaints) have been mild and self-resolving requiring symptomatic medications or dose-adjustments. (Leatherman *et al.*, 2007)

The safety of this technique will likely provide increased opportunities for higher risk patients, such as asthmatic persons and children under the age of 5. (Leatherman *et al.*, 2007 & Panjo 2007). Furthermore, the effectiveness of this method in allergic rhinitis and asthma and also for prevention of new sensitizations is noteworthy. (Milani *et al.* 2008).(12)

Besides the economic considerations, cultural level is also a determining factor for the decision to perform SLT and there is evident need for educational programs in this regard. (Ciprandi *et al.*, 2008). (13) The globally growing anti-vaccination movement is also discouraging patients from accepting SIT and certainly informing physicians about these associations will help overcome this problem. (Behramann, 2010). Developing biomarkers that determine patients' responsiveness to SLT and also showing clinical effectiveness of this modality, would also be beneficial. (Bullens, 2010).

Many different mediators and cytokines may initiate and partially sustain the inflammation of airways during an asthmatic response. Nitric oxide (NO) is produced by NO synthetase in various airway cells and may amplify and perpetuate allergic inflammation (Ricciadrolo, 2003) and its concentration in exhaled air and sputum is elevated in patients with asthma. SO, measurement of fractional exhaled nitric oxide (FENO) is a useful biomarker for assessment airway inflammation, and its technique is simple, noninvasive, affordable and applicable, and it is beneficial in the diagnosis of asthma and follows up of responsiveness to the treatment. But there is poor consistency among different studies on the threshold or cut-off levels, and the upper limits of normal FENO levels vary among different studies. (Samy *et al.*, 2010)

Eosinophil infiltration is also a characteristic feature of airways in asthma and elevated levels of eosinophils in blood and eosinophil cationic protein (ECP) in serum may be useful markers of airway inflammation in this disease. SO, ECP measurement provides good evidence for diagnosis of asthma. (Khakzad *et al.*, 2009).

The aim of our study is to investigate the efficacy of SLIT against *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, the main sources of house dust mite allergens for asthmatic children, on 2 immunologic parameters: ECP and NO concentrations. We also compared the relation to asthma symptom and medication scores, allergen-specific bronchial challenge test findings, and skin prick test findings.

Patients And Methods:

Thirty-six patients (17 females, 19 males) Children who attended the outpatient clinic with allergic mild-to-moderate asthma were studied prospectively. The asthma diagnosis was made according to American Thoracic Society criteria .The patients were monosensitized to house dust mites (*Dermatophagoides*

pteronysinus and *Dermatophagoides farinae*). As shown by a positive skin prick test and elevated levels of specific IgE to house dust.mite were included in this study. The mean (\pm SD) age of patients was 9.4 ± 2.5 years (range, 6-15 years). All subjects were offered SLIT.

The 19 patients who accepted underwent a $2 \pm$ year course of SLIT that was completed in all cases (SLIT group) and the remaining 17 subjects who declined SLIT (because of individual problems or fear of the medication, etc) served as the control group. The study was performed with the approval of all patients and informed consent was obtained from all patients. In our outpatient allergy clinic the number of acute asthma attacks reported by the patient is recorded at the time of diagnostic interview, before any anti-inflammatory medication is started. An attack is defined as cough, wheeze, and dyspnea that persisted for more than 24 hours and that were resolved with bronchodilator treatment. If the severity of asthma is mild, moderate or severe and persistent the patients are treated with inhaled budesonide. The study groups were comparable for age, asthma severity, lung function, and bronchial challenge test findings.

The clinical efficacy of SLIT was assessed by symptom and medication scores [22] and changes in lung function test parameters. Lung function tests, skin prick tests, and a bronchial challenge with *D pteronyssinus* were performed before and after 2 year of SLIT. Total serum IgE, specific IgE, serum ECP and serum NO levels were measured at baseline and after 2 year of SLIT.

A skin prick test was performed on each patient with the most common aeroallergen solutions (Stallergenes SA, Antony Cedex, France). A multi-test applicator (Hollister- Stier Laboratories, Spokane, Washington, USA) was used during the procedure. A wheal diameter of more than 3 mm was accepted as positive.

The patients visit the outpatient clinic every 3 months and a pediatric allergist records the number of acute asthma attacks lasting more than 24 hours and resolved with bronchodilator treatment, as noted in the patient's diary cards. If the patient is having an acute attack at the time of the routine visit, as detected during the pediatric allergist's examination, and in patient's file. After at least 6 months of using anti-inflammatory medication and allergen avoidance, if patient's symptoms are not completely controlled, pulmonary function tests are performed and SLIT is prescribed for patients who have a forced expiratory volume in 1 second (FEV1) above 70 %. The patients receive SLIT for 2 years. At the same time, anti-inflammatory medication is also prescribed and the dose is arranged according to clinical progress as noted in visits once in every 3 months with the pediatric allergist.

When the patient does not have any acute asthma attacks during the previous year, the anti-inflammatory medication is stopped. When the patient still does not have any symptoms for at least 6 months with no anti-inflammatory medication, they are accepted as being in complete remission. The main outcome measures for this study of patients undergoing those procedures were the number of acute asthma attacks that had been recorded before and after SLIT and the rate of complete remission.

A standardized extract of house dust mites (50% *D pteronyssinus*/50 % *D farinae*) (Stallergenes) was used. Twenty drops of the solution (100 index of reactivity [IR]) was placed under the tongue for 3 minutes on 3 alternate days a week. The comparison of the number of acute asthma attacks before and after SLIT was made with the Wilcoxon signedrank test.

All patients were given questionnaires after twenty four months treatment. Analysis of the efficacy of treatment showed that immunotherapy significantly improved the signs and symptoms of the group. Two mls of peripheral blood was drawn from the cubital vein of each subject in the study and let the blood clot at room temperature then all samples were centrifuged at 1000xg for 10 minutes at room temperature. The serum was collected and divided into 2 aliquots. The 1st aliquot stored at -20°C until assay of ECP. After thawing, serum samples were tested with immulite ECP (DPC, Los Angeles, USA) at the immulite 2000 automated analyzer (DPC, USA). Immulite ECP is a solid-phase, two site chemiluminescent immunometric assay (De Arruda, 2002). The 2nd aliquot were deproteinized with sulfosalicylic acid (35%). Then, nitrate (N03) in a supernatant was reduced by cadmium column (100 meshes) to nitrite (N02) and the concentration of N02 was determined with the Griess reaction (Mallorqui *et al.* 2000).

RESULTS AND DISCUSSIONS

Table 1: Study Design.

Items	Asthmatic group	Control group
Gender	9 ♀, 10 ♂	8 ♀, 9 ♂
Age (years)	9.4 ± 2.5	10.1 ± 2.2
Asthma severity	5 mild intermittent 6 mild persistent 8 moderate	

Table 2: Symptom and medication scores in all studied groups before and after drug therapy. (Mean± SD).

Items	Asthmatic group	Control group
Symptom Score		
- baseline	3.1± 0.9	3.3± 0.8
- after therapy	1.1±0.7*, **	1.5± 1.1
Medication Score		
- baseline	4.9± 1.4	5.3± 1.9
- after therapy	2.1± 1.3***	2.5± 1.8****

* P < 0.05: versus control group after therapy

**P< 0.001: versus baseline of asthmatic group.

***P< 0.01: versus baseline of asthmatic group.

****P< 0.05: versus baseline of control group

Table 3: Lung function tests in all studied groups before and after drug therapy. (Mean± SD).

Parameters	Asthmatic group	Control group	P
FVC,% of predicted			
- baseline	83.1± 16.2	81.2±8.1	< 0.05
- after therapy	88.0± 14.8	90.4± 7.3	< 0.05
FEV ₁ ,% of predicted			
- baseline	83.8± 17.1	80.57± 4.7	< 0.05
- after therapy	80.3± 12.3	88.9± 5.6	< 0.01
PEF, % of predicted			
- baseline	77.3± 12.1	88.9± 6.4	< 0.01
- after therapy	89.4± 13.5	92.6± 7.8	< 0.05
MEF ₂₅₋₇₅ , % of predicted			
- baseline	110.3± 24.6	105.0± 23.1	< 0.05
- after therapy	108.4± 21.8	100.1± 21.3	< 0.001

FVC: Forced vital capacity.

EFV₁: Forced expiratory volume in 1 second.

PEF: Peak expiratory flow.

MEF₂₅₋₇₅: Forced mid-expiratory flow at 25% to 75% of FCV.

Table 4: Serum ECP and serum NO levels in all studied groups before and after therapy. (Mean± SD).

Parameters	Asthmatic group	Control group	P
Serum ECP(ng/mL)			
- baseline	28.9± 3.4	9.3± 4.3	< 0.001
- after therapy	15.9± 2.8	9.1± 4.1	< 0.001
Serum NO(µmol/L)			
- baseline	60.1± 8.7	28.4± 2.1	< 0.001
- after therapy	25.8± 3.6	22.4± 1.4	< 0.001

Discussion:

Immunotherapy is the medical term for a treatment for asthma commonly known as "Allergy shots." It can help reduce asthma symptoms, improve pulmonary functions and the need for medications in people who are allergic to airborne allergens, such as pollen, pet dander, and dust mites. Abramson *et al.*, (2006).

So, Immunotherapy may prevent the onset of asthma, and is also an effective treatment for asthma. Five years of age is the youngest recommended age to start immunotherapy in the United States; younger children may be in-cooperating with the immunotherapy program. There is no upper age limit for receiving immunotherapy. In considering immunotherapy attention must be given to the other medical conditions (such as cardiac disease) that are more frequent in older individuals, which could potentially make immunotherapy more risky. Failure to respond to immunotherapy may be due to several factors including: inadequate dose of allergen in the allergy vaccine, missing allergens not identified during the allergy evaluation, high levels of allergen in environment (i.e. inadequate environmental control), significant exposure to non-allergic triggers (i.e. tobacco smoke) (Senti *et al.*, 2010).

Our results confirm that SLIT is effective for treating asthmatic children sensitized to house dust mite. This study demonstrated that SLIT with *D pteronyssinus* and *D farinae* extracts appeared to improve clinical symptoms, and indices of allergic inflammation as determined by serum NO, and ECP concentrations in children who received SLIT when compared to the control group. In addition, we also found that unlike the control group, the SLIT group showed a tendency for skin reactivity to mite to decrease, although the change did not reach statistically significance.

Nitric oxide (NO), plays an important role in physiologic airway regulation, is synthesized from L-arginine by NO synthetase. Three isoforms of NO synthase are known and both the first and the third are constitutively expressed in the human airway, whereas the second is inducible by inflammatory stimuli - *Barnes et al., (1995)*. (Fractional exhaled nitric oxide (FENO) is a marker of airway inflammation and is increasing during periods of uncontrolled asthma and reduced during treatment with anti-inflammatory agents Silkoff *et al.,* 2004).

In addition, it has also been reported that asthmatic patients have higher levels of NO in peripheral blood and that serum levels can be used as an additional inflammatory marker in asthma Verhagen *et al.,*(2005). No study has yet to investigate the effect of SLIT on serum NO concentration, although SLIT with *D pteronyssinus* and *D Farinae* extracts has been found to reduce exhaled NO in asthmatic children with mite allergy and to lead to hypo sensitization Dc Arruda *et al.,* (2002). Contrary to those findings, in this study, serum NO levels decreased after SLIT, possibly reflecting a reduction in systemic allergic reaction.

Activated eosinophils release various cytotoxic proteins such as ECP, arachidonic acid metabolites, and oxygen-derived radicals. ECP has been considered to have an important role in the pathogenesis of allergic diseases. (Mallorquí *et al.,* 2000) suggested that the severity of bronchial obstruction in asthmatic patients might be estimated by ECP concentration in sputum. (Jönsson *et al.,* 2010) showed the associations of symptoms of allergy and asthma to ECP-genotypes .However (Noguchi *et al.,*2003) reported that serum ECP levels were not elevated in some patients with asthma, even when they were symptomatic. (Bartoli *et al.,* 2004) revealed that sputum ECP in different stages of untreated asthma was uniformly elevated. Therefore ECP in serum and sputum are a reliable test for diagnosis of asthma. McCormack *et al.,* (2008)

Serum levels of ECP have thus been considered useful to monitor airway inflammation in asthma as it correlates with sputum eosinophil counts Hung *et al.,* (2004) Increased ECP release from airway mucosa cells has been said to occur in parallel with raised serum ECP levels and SLIT has been shown to decrease serum ECP Wang *et al.,* (2006). In this study, serum ECP concentration decreased significantly in the SLIT group, but did not change in the pharmacotherapy group, consistent with these previous studies.

Conclusion:

We found that house dust mite immunotherapy was effective in improving immunological and clinical parameters in asthmatic children. Our study confirmed an anti-inflammatory effect of SLIT evidenced by its decreasing the serum concentration of inflammatory mediators (ECP and NO). Skin prick tests may be used as a marker of efficacy of therapy.

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