The Egyptian Journal of Hospital Medicine (July 2019) Vol. 76 (6), Page 4386-4389

Laboratory Evaluation of Immunotherapy in Treatment of Allergic Airway Diseases

Mohamed Saed Elshorbagy[1], Ismael Abdelmonem Atia[2],
Nagah Mohamed Abou-Mohamed[1] and Abdelsalam Fathy Abdelsalam Mohamed[1]*
1-Immunology Unit of Clinical Pathology Department, 2- Chest Diseases Department,
Faculty of Medicine, Al-Azhar University
***Correspondence author**: Abdelsalam Fathy Abdelsalam Mohamed, **Mobile:** (+20) 01016704537,
E-mail: dr_abdelsalam2000@yahoo.com

ABSTRACT

Background: Immunotherapy is a well-established therapeutic approach for treatment of IgE-mediated allergic diseases. It's the only modality that targets the definite cause of allergy, blocks the pathophysiologic aspect of the disease and possibly prevents the development of a new sensitization. **Objective:** The aim of this work is evaluation of the response of allergic airway diseases to allergen specific immunotherapy. **Patients and methods:** We enrolled 50 patients with allergic airway diseases receiving subcutaneous immunotherapy (SCIT) shots and 30 control patients on pharmacotherapy. The study evaluated clinical assessment of allergic symptoms, medication used and laboratory markers (including specific and total IgE and eosinophil count) before and after 2 years of immunotherapy. **Results:** Our results showed that there was a significant decrease in total and specific IgE and eosinophil count after SCIT. Also, SCIT effectively reduced the allergic symptoms and the need for medication.

Conclusion: Subcutaneous immunotherapy could alleviate allergic symptoms and reduce airway inflammation in allergic patients.

Keywords: allergic airway diseases, allergy immunotherapy, allergy injections, subcutaneous immunotherapy, IgE.

INTRODUCTION

Respiratory allergies are a major health problem in both developed and developing countries. Over the past four decades, there has been a significant increase in the prevalence of allergic disease. Respiratory allergies are the most common chronic diseases among adolescents and young people. The increase is particularly problematic in children [(1)].

Asthma is a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness and airflow obstruction. Allergic rhinitis implies a blocked or runny nose, sneezing, and itching secondary to immunoglobulin IgE-mediated inflammation of the nasal mucosa. Rhinitis often occurs in combination with conjunctivitis. The genetic predisposition to develop IgE-mediated sensitivity to common aeroallergens is the strongest predicting factor for the development of rhinoconjunctivitis as well as asthma. The allergic reaction is biphasic, with an immediate reaction occurring within minutes following allergen exposure and a late-phase reaction occurring hours later [(2)].

Clinical guidelines recommend a combination of patient education, allergen avoidance, pharmacotherapy, and allergy immunotherapy for treatment. Drugs are available for the treatment of allergic symptoms, but many patients report insufficient symptom control. Importantly, pharmacotherapy has no effect on the progression of the disease and treatment has to be administered repeatedly as long as symptoms prevail, which often means life-long [(3)].

Allergy immunotherapy is a causal treatment targeting the underlying allergic disease, affecting basic immunological mechanisms and resulting in the induction of immunological tolerance. Induced tolerance implies disease modification, the clinical effects of which are sustained symptom relief after completed treatment and/or prevention of disease progression. The capacity to alter the natural course of the disease differentiates allergy immunotherapy from other treatment modalities. Therefore, spending time, effort, and money on immunotherapy represents an investment that will return sustained benefits from improved prognosis and a relieved burden of disease. The future aim for allergy immunotherapy is to expand the evidence base concerning the benefits of disease modification [(4)].

AIM OF THE WORK

The aim of this work is evaluation of clinical and some laboratory markers of allergy in allergic patients (bronchial asthma + allergic rhinitis) under subcutaneous allergen specific immunotherapy for 2 years.

PATIENTS AND METHODS

This study was performed on eighty (80) persons with allergic airway diseases (bronchial asthma ± allergic rhinitis), with mean age 27 years. They were divided into 2 main groups: Fifty (50) allergic patients as a case group (Group 1) were treated by immunotherapy and pharmacotherapy and thirty (30) allergic patients of matched age and sex as control group (group 2) treated by pharmacotherapy only.

Study design

Patients were recruited from Chest Clinics in Al-Hussein University Hospital and received the immunotherapy and investigated at Immunology Unit in the Clinical Pathology Department.

Ethical approval:

Research participants were selected randomly as regard identification, age, sex.

The study was approved by the Ethics Board of Al-Azhar University and an informed written consent was taken from each participant in the study.

Received:11/5/2019
Accepted:10/6/2019

All patients were allowed to continue their ordinary medication in the form of inhaled steroids, bronchodilators and antihistaminic.

Group 1 and group 2 were evaluated for diagnosis and severity of allergy before and after immunotherapy according to GLOBAL INITIATIVE FOR ASTHMA (GINA 2016).

Inclusion criteria:

• Asthmatic ± allergic rhinitis patients according to GINA 2016 guidelines.

• Atopic patients proved by positive skin prick test and elevated serum IgE.

• Skin prick test confirming polysensitization to multiple allergens.

Exclusion criteria:

• Severe persistent asthma [assessed by clinical symptoms according to GINA (2016)].

• Skin prick test showing sensitization to a single allergen.

• Chest x-ray suggestive of any associated bronchopulmonary disorders.

• Pregnant or lactating patient.

• Smokers.

• Patients on allergy immunotherapy (AIT) before the start of the study.

METHODS

For all enrollees, skin prick test was done and the following tests were done before and after 2 years of immunotherapy, complete blood picture (CBC), total IgE and specific IgE for HDM (house dust mites). We selected our cases according to the result of the skin prick test which was done using extracts from STALLEGENES GREER Company, USA for allergy testing products. Skin prick test was done for 177 enrollees to confirm sensitization to multiple allergens and to identify allergens for which they had to receive immunotherapy. The test panel consisted of most common allergen extracts which are prevalent in the local environment like mixed molds, house dust mite, candida, hay dust, cat hair, mixed pollen, wool, latex, tobacco, dog hair, feather, cotton dust and cockroaches.

We selected 80 cases who were highly positive to house dust mite (HDM) mainly with area of wheal and flare >= 3 ml more than negative control. The cases were weekly positive to allergens other than HDM but not the same in all cases, so some cases were +ve to mite, tobacco and Aspergillus others were +ve to mite, Alternaria and ragweed and so on.

Table (1): results of skin prick test.

Mite p	Cockroach	Ragweed
80/80	11/80	33/80
Mite F	Alternaria	Wool
80/80	21/80	8/80
Cat D	Aspergillus N or F	Molds
19/80	27/80	14/80
Tobacco	Grass pollen	
15/80	12/80	

Samples:

Under complete aseptic condition, 8 ml of venous blood were withdrawn from the antecubital veins with a 20-gauge needle. All precautions were taken to avoid hemolysis, so hemolysed samples were rejected. The blood sample was then divided into 2 tubes with the following order:

1- Two ml was collected into vacutainer tube containing K_3-EDTA for complete blood count and eosinophilic count. CBC analysis was done on [Sysmex XS500] Japan.

2- Five ml venous blood sample on plain tube prepared for separation of sera which used for the following tests:

Total IgE was done by enzyme linked immune sorbent assay (ELISA) technique using commercial kit RIDASCREEN® (Article no.: A0141) from R-Biopharm Company (Germany).

Specific IgE for HDM was done by ELISA technique using commercial kit RIDASCREEN® (Art. No.: A0041) from R-Biopharm Company (Germany).

Immunotherapy protocol

The allergen extracts we used is that of STALLEGENES GREER Company USA Allergy Products. The allergens selected for immunotherapy were the allergens that showed positivity by skin prick test. The main extracts used for multi-allergen immunotherapy (AIT) were dust mites, ragweed, aspergillus, alternaria, pollens, animal dander, molds, tobacco, wool and cockroach. We avoided mixing of molds or cockroach extracts with pollen extracts as the former extracts (molds and cockroach) tend to have high proteolytic enzyme activities. Group 1 received subcutaneous immunotherapy (SCIT) using the 5 most clinically relevant allergens identified for each patient according to both history and skin prick test results but not more than 5 allergens to avoid increased dilution that would prevent the delivery of the optimal dose of each allergen. Immunotherapy was administered for each patient with 10-fold increase in concentration between each bottle (1/10000, 1/1000, 1/100,). Increasing volumes (0.2, 0.4, 0.6 and 0.8 ml of each vial) of allergen extracts were injected subcutaneously ranging from 3 times to twice weekly for the first 4 bottles for 4-6 months (Build up). After which 0.5 ml of vial number 5 was administered every 2 weeks to every month as maintenance treatment. Patients who were unable to tolerate higher concentrations due to local or systemic reactions were maintained on the highest concentration they were able to tolerate. The complete regimen lasts 2-5 years according to the clinical response of the patient. The buildup phase of immunotherapy was extended in some patients to 6 -8 months [5].

Statistical methods

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance: was used when comparing between two means.
- Chi-square test: was used when comparing between qualitative data.
- Probability (P-value). P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value >0.05 was considered insignificant.

RESULTS

There was a highly statistically significant difference between absolute eosinophil count (before and after therapy) in patients group while no significant difference between absolute eosinophil counts (before and after therapy) in control group (table 2).

Table (2): Comparison of absolute eosinophil count (before and after therapy) in studied groups.

Groups \ Variables		Abs. eosinophil count /mm^3		p-value
		before	After	
Patients (N = 50)	Mean	402.00	328.00	< 0.001*
	±SD	13.98	88.16	
Control (N = 30)	Mean	386.67	396.67	0.679
	±SD	10.80	85.03	

Also, a statistically significant difference between total IgE (before and after therapy) in patients group and no significant difference between total IgE (before and after therapy) in control group (table 3).

Table (3): Comparison of Total IgE (before and after therapy) in studied groups.

Groups \ **Variables**		**Total IgE. IU/ml**		**p-value**
		before	After	
Patients (N = 50)	Mean	218.24	187.52	0.004**
	±SD	55.15	9.37	
Control (N = 30)	Mean	199.73	209.20	0.199
	±SD	27.53	28.89	

Highly statistically significant difference between specific IgE (before and after therapy) in patients group with no significant difference between specific IgE (before and after therapy) in control group (Table 4).

Table (4): Comparison of Specific IgE (before and after therapy) in studied groups.

Groups \ **Variables**		**Specific IgE. IU/ml**		**p-value**
		before	After	
Patients (N = 50)	Mean	0.76	0.59	< 0.001*
	±SD	0.09	0.18	
Control (N = 30)	Mean	0.68	0.70	0.447
	±SD	0.09	0.08	

Improvement in patients receiving immunotherapy was noticed and evaluated clinically according to GINA 2016, statistically significant difference between clinical symptoms (before and after therapy) in patients group was noticed while slight improvement was noticed in patients on medical therapy with non-statistically significant difference before and after therapy in control group (Table 5).

Table (5): Comparison of clinical symptoms (before and after therapy) in studied groups.

Groups \ **Variables**		**Clinical symptoms**		**p-value**
		before	After	
Patients (N = 50)	No Sym.	0 (0%)	5 (10%)	< 0.001*
	Mild Sym.	15 (30%)	35 (70%)	
	Moderate Sym.	13 (26%)	7 (14%)	
	Severe Sym.	22 (44%)	3 (6%)	
Control (N = 30)	Mild Sym.	10 (33.3%)	13 (43.3%)	0.414
	Moderate Sym.	12 (40%)	13 (43.3%)	
	Severe Sym.	8 (26.7%)	4 (13.3%)	

- There was no association between clinical improvement and studied laboratory parameters (eosinophil count, Total IgE and specific IgE) after 2 years of immunotherapy.

DISCUSSION

Allergic airway disease is a global health problem that causes significant illness and disability worldwide. Interaction between lower and upper airways are well known and have been studied since1990. More than 80% of asthmatics have rhinitis and 10 – 40% of patients with rhinitis have asthma. Indoor and outdoor allergens, as well as occupational agents, lead to allergic airway disease [(6)].

The mechanism of immunotherapy entails redirection of the T lymphocyte response from a T helper cell type 2 phenotype by induction of regulatory T cells and/or immune deviation toward a T helper cell type 1 phenotype, with resulting inhibition of downstream effector pathways and induction of immunoglobulin G-associated blocking antibodies [(7)].

In our study, we found that AIT for HDM administered by the SCIT route is effective in reducing clinical manifestations, and laboratory markers (eosinophilic count, total IgE and specific IgE for house dust mites). These findings are going with the results of other studies **Stylianou *et al*.[(4)], El Shayeb *et al*.[(8)], Zielen *et al*.[(9)] and Marappan[(10)]** and contradictory to studies done by **Levin *et al*.[(11)], Karakoc-Aydiner *et al*.[(12)], Hoon *et al*.[(13)] and Rosa *et al*.[(14)]** who stated that there is no change in laboratory markers in spite of clinical improvement.

These discrepancy in findings of different studies may be attributed to differences between the reported studies in: (i) the number of allergen extracts used (either single or multiple); (ii) the type of allergen products (homemade or commercial); (iii) the total duration of immunotherapy administration; (iv) the different routes of administration and (v) timing of lab. parameters evaluation.

In our study, there was no association between clinical improvement and studied laboratory parameters (eosinophil count, total IgE and specific IgE) after 2 years of SCIT in patients group. This result goes with the studies done by **Rosa *et al*. [(13)], Shamji and Durham[(15)], Nouri-Ari *et al*.[(16)] and Pilette *et al*.[(17)]** and contradictory to the studies done by **Ciprandi and DeAmici[(18)]** and **Rolinck-Werninghaus *et al*.[(19)].** This discrepancy could be attributed to failure of SCIT to overcome hyperirritability state associated with allergy.

CONCLUSION

AIT has the potential to achieve improvement in clinical manifestations and some laboratory markers (absolute eosinophil count, total IgE and specific IgE). There is no association between clinical improvement and done lab. markers.

REFERENCES

1. **Tomasiak-Łozowska MM, Klimek M, Lis A *et al.* (2018):** Markers of anaphylaxis - a systematic review. Adv Med Sci., 63: 265-277.
2. **Yukselen A, Kendirli SG, Yilmaz M *et al.* (2012):** Effect of one-year subcutaneous and sublingual immunotherapy on clinical and laboratory parameters in children with rhinitis and asthma: a randomized, placebo-controlled, double-blind, double-dummy study. Int. Arch. Allergy Immunol., 157(3), 288–298.
3. **Chelladurai Y, Suarez-Cuervo C, Erekosima N *et al.* (2013):** Effectiveness of subcutaneous versus sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. J Allergy Clin Immunol Pract., 1(4):361-9.
4. **Stylianou E, Thor U, Fredrik B *et al.* (2016):** Specific allergen immunotherapy: effect on IgE, IgG4 and chemokines in patients with allergic rhinitis. Scandinavian Journal of Clinical & Laboratory Investigation, 76(2): 118-12.
5. **Cox L, Harold N, Richard L *et al.* (2011):** Allergen immunotherapy: A practice parameter third update. J Allergy Clin Immunol., 127:1-55.
6. **Khaled E, Salama E, Ezzat R *et al.* (2018):** Prevalence of allergic disorders of respiratory system in children in Shebin Elkom. Journal of Medicine in Scientific Research, 4: 1-13.
7. **Pajno GB, Fernandez-Rivas M, Arasi S *et al.* (2018):** EAACI Guidelines on allergen immunotherapy: IgE-mediated food allergy. Allergy, 73:799-815.
8. **El Shayeb M, Dina SS, Asmaa SA *et al.* (2015):** The Efficacy of Single versus Multiple Allergen Immunotherapy in Polysensitized Asthmatic Patients: A Double-blinded Randomized Clinical Trial. International Trends In Immunity, 3: 2326-3121.
9. **Zielen S, Kardos P, Madonini E (2010):** Steroid-sparing effects with allergen-specific immunotherapy in children with asthma: a randomized controlled trial. J. Allergy Clin. Immunol., 126(5): 942–949.
10. **Marappan M (2008):** Changes In Clinical Status And Serum Ige Levels Following Sublingual Immunotherapy For 2 Years In Patients With Allergic Rhinitis and/or Asthma Due To House Dust Mite. Chest, 134(4): 1-8.
11. **Levin M, Jasmine JK, Jacob G *et al.* (2015):** Persistence and evolution of allergen-specific IgE repertoires during subcutaneous specific immunotherapy. J Allergy Clin Immunol., 137(5): 1535–1544.
12. **Karakoc-Aydiner E, Eifan AO, Baris S *et al.* (2015):** Long-Term Effect of Sublingual and Subcutaneous Immunotherapy in Dust Mite–Allergic Children With Asthma/ Rhinitis: A 3-Year Prospective Randomized Controlled Trial. J Investig Allergol Clin Immunol., 25(5): 334-342.
13. **Hoon SK, Seung YS, Kun HL *et al.* (2014):** Long-term Effects of Specific Allergen Immunotherapy against House Dust Mites in Polysensitized Patients With Allergic Rhinitis. J Allergy Asthma Immunol Res., 6:535-540.
14. **Rosa DG, Luigi C, Saverio N *et al.* (2000):** Effects of Specific Immunotherapy in Allergic Rhinitic Individuals with Bronchial Hyperresponsiveness. American Journal of Respiratory and Critical Care Medicine, 16(4*)*:793.
15. **Shamji MH, and Durham SR (2017):** Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers J Allergy Clin Immunol., 140(6):1485-1498.
16. **Nouri-Aria KT, Wachholz PA, Francis JN *et al.* (2004):** Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol., 172:3252-9.
17. **Pilette C, Nouri-Aria KT, Jacobson MR *et al.* (2007):** Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. J Immunol., 178:4658-66.
18. **Ciprandi G and DeAmici M (2014):** Serum IgE assessment in prescribing allergen immunotherapy. Ann Allergy Asthma Immunol., 112 (2014) 184-185.
19. **Rolinck-Werninghaus C, Keil T, Kopp M *et al.* (2008):** Specific IgE serum concentration is associated with symptom severity in children with seasonal allergic rhinitis. J Allergy, 63: 1339–1344.