The Efficacy of Single versus Multiple Allergen Immunotherapy in Polysensitized Asthmatic Patients: A Double-blinded Randomized Clinical Trial

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Abstract — Background: Immunotherapy has been used excessively in treatment of allergic asthma. There is much debate as to whether polysensitized patients are best treated with several allergens or the single most clinically problematic allergen. Objective: This study aimed to assess the efficacy of single versus multiple allergen subcutaneous immunotherapy in polysensitized asthmatic patients. Methods: This randomized double blinded clinical trial included 60 atopic asthmatic patients attending the outpatient clinic of allergy and clinical immunology at Ain Shams University hospital. After performing skin prick test to common aeroallergens, patients were divided into two groups: Group A included 30 patients who received single allergen immunotherapy using the most clinically relevant allergen. Group B included 30 patients who received multiple allergen immunotherapy. Both groups received immunotherapy for 6 months. Asthma severity score, asthma control test, FEV1 as well as total IgE and interleukin 4 were assessed before and after the treatment period. Results: Both groups showed improvement across all test parameters. Group A treated with single allergen immunotherapy showed a more significant drop in total serum IgE. Conclusion: Single allergen immunotherapy might be more effective than multiple allergen immunotherapy as evidenced by decrease in serum total IgE and IL-4 secretion.

Keywords — allergic asthma, poly-sensitization, immunotherapy, interleukin-4, serum IgE.

INTRODUCTION

Allergic asthma results from a type I hypersensitivity reaction with the release of several mediators that directly contract airway smooth muscle causing acute bronchoconstriction [1]. Interleukin (IL-4) is a key cytokine in the development of allergic inflammation through secretion of IgE by B lymphocytes [2]. Specific immunotherapy has been used excessively in treatment of allergic asthma and a large number of clinical studies demonstrated significant improvement in both children and adults. Benefits included a decrease in asthma symptoms and need of medical treatment, improvement in pulmonary function test and reduction in both nonspecific and allergen specific bronchial responsiveness [3]. Poly-sensitization is more prevalent than mono-sensitization in patients with atopic asthma. There is much debate as to whether polysensitized patients are best treated with several allergens or the single most clinically problematic allergen [4]. This study aimed to assess the efficacy of single versus multiple allergen subcutaneous immunotherapy (SCIT) in polysensitized asthmatic patients.

I. MATERIALS AND METHODS

This randomized double blinded clinical trial included 60 atopic asthmatic patients attending the outpatient clinic of allergy and clinical immunology at Ain Shams University hospital during the year 2012. The study was approved by the Ain Shams medical research ethics committee, and informed consent was obtained from the patients prior to enrollment. All patients were diagnosed according to the global initiative for asthma management and prevention (GINA 2011) [5]. Inclusion and exclusion criteria are shown in table (1).
Table 1: Inclusion and exclusion criteria.

Inclusion criteria:
- Asthmatic patients according to GINA 2011 guidelines.
- Atopic patients proved by positive skin prick test and high serum IgE.
- Skin prick test confirming polysensitization to several allergens.

Exclusion criteria:
- Mixed allergy (asthma associated with rhinitis and/or skin allergy).
- Severe persistent asthma [assessed by PFT according to GINA (2011)].
- Skin prick test showing sensitization to a single allergen.
- Chest x-ray suggestive of associated bronchopulmonary disorders.
- Pregnant or lactating females.
- Smokers.
- Patients on IT before the start of the study.

Skin prick test was done to confirm sensitization to multiple allergens and to identify allergens for which they will receive immunotherapy. The test panel was prepared at the Allergy and Clinical Immunology Unit laboratory at Ain Shams University hospital and it comprised of 26 most common allergen extracts which are prevalent in the local environment (table 2). The allergens selected for immunotherapy were the allergens that showed strong positivity by skin prick test. The main extracts used for multi-allergen immunotherapy (AIT) were pollens, animal dander, dust mites, molds, 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. No food allergens were used for AIT.

Table 2: Inlet criteria

<table>
<thead>
<tr>
<th>Inhalants</th>
<th>Ingestants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed molds</td>
<td>House dust</td>
</tr>
<tr>
<td>Mite</td>
<td>Tobacco</td>
</tr>
<tr>
<td>Candida</td>
<td>Pigeon</td>
</tr>
<tr>
<td>Hay dust</td>
<td>Dog hair</td>
</tr>
<tr>
<td>Cat hair</td>
<td>Feather</td>
</tr>
<tr>
<td>Mixed pollen</td>
<td>Cotton dust</td>
</tr>
<tr>
<td>Wool</td>
<td>Cockroaches</td>
</tr>
<tr>
<td>Eggs</td>
<td>Strawberry</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>Cocoa</td>
</tr>
<tr>
<td>Apricot</td>
<td>Banana</td>
</tr>
<tr>
<td>Fish</td>
<td>Wheat</td>
</tr>
<tr>
<td>Milk</td>
<td>Maize</td>
</tr>
</tbody>
</table>

Patients were randomly divided into two groups using block randomization with a block size of 4. Group A included 30 patients who received single allergen immunotherapy using the single most clinically relevant allergen identified for each patient according to both history and skin prick test results.

Group B included 30 patients who received multiple allergen immunotherapy. The number of allergens used in the mixture prepared for immunotherapy varied depending on the potential interactions between different types of allergens, with a maximum number of 5 allergens to avoid excessive dilution that would interfere with the delivery of the optimal dose of each allergen. We avoided mixing of allergens that could lead to degradation or unmasking of epitopes on exposure to proteolytic enzymes.

Extracts were prepared as an aqueous solution using the weight/volume (wt/vol) unit. A potency of 1:100 indicates that 1 g of dry allergen was added to 100 cc of a buffer (phenol saline) for extraction. The allergen was eluted for a time, and then the solid material was filtered out, leaving an aqueous solution [6]. Immunotherapy was administered for each patient with 10-fold increase in concentration between each bottle (1/1000, 1/100, 1/10). Increasing volumes (0.2, 0.4, 0.6, 0.8, and 1ml of each vial) of allergen extracts were injected subcutaneously twice weekly for 3 months, after which 1ml of vial number 3 was administered weekly 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.

Each patient was assessed using asthma control test (ACT) (score ≤19 indicates uncontrolled asthma) [7], spirometry, asthma severity score (Table 3) [8], total serum IgE, and serum IL-4. Pulmonary function tests were performed using computerized spirometer (MasterlabYaeger, Wurtzburg, Germany). Forced expiratory volume 1 (FEV1) and forced vital capacity were the parameters used in this study. Serum IgE and IL-4 were measured using ELISA.

These tests were performed as a baseline before the start of immunotherapy and after 6 months of treatment.
Total IgE assay

Total serum IgE levels were measured using IgE Accubind ELISA (Monobind, Inc. Lake Forest, CA, USA) according to the manufacturer’s instructions. This procedure has a sensitivity of 1 IU/ml. The normal level of total IgE, in adults, is less than 100 IU/ml. The intraassay and interassay coefficients of variation were 3.9% and 5.2%, respectively.

IL-4 assay

Detection and quantitative measurement of IL-4 in serum were done by AviBion Human IL-4 ELISA kit (Orgenium Laboratories, Finland). The minimum detectable concentration (MDC) was estimated to be 2 pg/ml. The intraassay and interassay coefficients of variation for IL-4 were 4.7% and 5.2%, respectively.

Table 3: Asthma severity classification [7].

<table>
<thead>
<tr>
<th>Components</th>
<th>Intermittent</th>
<th>Mild Persistent</th>
<th>Moderate Persistent</th>
<th>Severe Persistent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>≤ 2 days/week</td>
<td>&gt; 2 days/week</td>
<td>Daily</td>
<td>Throughout the day</td>
</tr>
<tr>
<td>Nighttime awakenings</td>
<td>≤ 2/month</td>
<td>3-4/month</td>
<td>&gt; 1/week but not nightly</td>
<td>Nightly</td>
</tr>
<tr>
<td>SABA use for symptoms control</td>
<td>≤ 2 days/week</td>
<td>&gt; 2 days/week but not daily ; not &gt; 1/day</td>
<td>Daily</td>
<td>Several times/day</td>
</tr>
<tr>
<td>Interference with normal activity</td>
<td>None</td>
<td>Minor limitation</td>
<td>Some limitation</td>
<td>Extreme limitation</td>
</tr>
<tr>
<td>Lung function</td>
<td>FEV1 &gt; 80% of predicted ; FEV1/FVC normal</td>
<td>FEV1 &gt; 80% of predicted ; FEV1/FVC normal</td>
<td>FEV1 60-80% of predicted ; FEV1/FVC reduced 5%</td>
<td>FEV1 &lt; 60% of predicted ; FEV1/FVC reduced &gt; 5%</td>
</tr>
</tbody>
</table>

Statistical analysis:

This was done on a personal computer using the Statistical Package for Social Sciences version 15 (SPSS©, SPSS Inc., Chicago, IL). Data were expressed as Mean± SD for quantitative parametric measures and both number and percentage for categorized data. Student’s t - test was used for comparison between two independent groups for parametric data. Paired t- test was used for comparison between two dependent groups for parametric data. A p value of less than 0.05 was regarded as statistically significant.

II. Results

Sixty asthmatic patients (9 males and 51 females) were included in the study. Their age ranged between 17 and 56 years, with a mean age of 33.03 years (±10.24 SD). All patients were atopic according to positive results of skin prick test.

Patients were randomly divided into two equal groups: group A received single allergen immunotherapy and group B received multiple allergen immunotherapy. All patients were given medical treatment for control of their asthma in the form of corticosteroids and short acting beta-agonist (SABA) inhalers.

None of the patients experienced any systemic side effects of the immunotherapy. 6 patients experienced a local reaction to the immunotherapy in the form of itching and rash

Eighteen patients stopped their medications completely in group A and three (10%) in group B. Nevertheless, there was no difference detected between pre- and post-immunotherapy spirometry results in both groups.

Table 3 shows classification of enrolled patients as regards asthma severity before and after immunotherapy. There was no statistically significant difference between both groups before and after immunotherapy as regards severity. Using the ACT as an indicator of uncontrolled asthma, both groups showed a statistically significant improvement in the mean score post-immunotherapy but there was no statistically significant difference between the two groups (table 4).

Table 4: Results of asthma control test (ACT) pre- and post-immunotherapy.

<table>
<thead>
<tr>
<th>ACT result</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean score before immunotherapy (SD)</td>
<td>16.1 (±3.67)</td>
<td>14.3 (±1.98)</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean score after 6 months (SD)</td>
<td>21.2 (±4.03)</td>
<td>18.3 (±2.5)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 5 shows the results of serum IgE and serum IL-4 pre- and post-immunotherapy. Both groups showed improvement in these parameters post-immunotherapy but the drop in serum IgE was more remarkable in group A who received single allergen immunotherapy. Although there was considerable difference in IL4 at baseline between group A and B, the reduction in IL4 after AIT was more pronounced in group A as
indicated by p-value which was 0.002 before AIT and was much lower after AIT p-value 0.0003.

Table 5: Results of Serum total IgE (IU/ml) and serum IL-4 (pg/ml) pre- and post-immunotherapy.

<table>
<thead>
<tr>
<th>Serum total IgE</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before immunotherapy (mean ±SD)</td>
<td>281.8±178.3</td>
<td>366.04±239.6</td>
<td>0.1</td>
</tr>
<tr>
<td>After 6 months (mean ±SD)</td>
<td>132.92±134.2</td>
<td>253.24±246.1</td>
<td>0.02</td>
</tr>
<tr>
<td>p value</td>
<td>0.01</td>
<td>0.08</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum IL-4</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before immunotherapy (mean ±SD)</td>
<td>8.3±9.3</td>
<td>15±6.49</td>
<td>0.002</td>
</tr>
<tr>
<td>After 6 months (mean ±SD)</td>
<td>3.35±1.9</td>
<td>8.1±6.39</td>
<td>0.0003</td>
</tr>
<tr>
<td>p value</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

III. DISCUSSION

Allergic asthma is the most common form of asthma. It is characterized by symptoms provoked by identifiable trigger to which the patient has been sensitized [9]. The pathogenesis of asthma is mediated by CD4+T-helper cells that produce type 2-cytokine profile. IL-4 and IL-13 appear to play a key role. IL-4 is critical in induction of IgE synthesis [2].

Specific immunotherapy is defined as method of administration of increasing amounts of specific allergen to patients with atopy, to modulate IgE-mediated sensitization to tolerate this specific allergen again [10]. There is a debate whether polysensitized patients are best treated with multiple allergens simultaneously or a single allergen per a vial. Immunotherapy with multiple allergens in polysensitized patients is a common practice in the United States, whereas in Europe the treatment with one or a few relevant allergens is preferred [4].

Most clinical trials assessing the efficacy of immunotherapy have been performed with a single allergen. Few studies have specifically addressed the effect of mixed allergens. Our study aimed at comparing the efficacy of single versus multiple allergen subcutaneous immunotherapy in poly-sensitized asthmatic patients.

In this study, application of single-allergen immunotherapy showed highly significant decrease in the levels of total IgE and IL-4, level was higher than in patients taking multi-allergen immunotherapy. This signifies the ability of single allergen immunotherapy in enhancing immunological control. Various studies observed a reduction in level of IL4 after single allergen [11] and multi-allergen [12]. However other studies couldn’t detect change in IL4 level after immunotherapy [13, 14].

IL-4 has been documented as one of the TH2 cytokines and has been found to be critical in the development of immediate hypersensitivity and necessary for IgE switch. Allergen immunotherapy affects the cytokine profile of allergen-specific T cells and shifts TH2-type immune responses in atopic individuals towards TH0 or TH1-type immune responses. There is however a discrepancy in findings of different studies regarding the level of IL-4. This may be attributed to differences between the reported studies in: (i) the stimulus used for in vitro restimulation, i.e. polyclonal or allergen-specific; (ii) the total duration of immunotherapy administration; and (iii) the different mechanisms in allergen-specific immunotherapy[15].

Regarding total IgE, similar results were achieved in a study on 14 children with positive skin prick test to Dermatophagoidespteronyssinus and Dermatophagoides farina. After receiving a total of 9 months of SCIT for house dust mites, there was a decrease in the level of total IgE at 4 and 9 months post-immunotherapy [16].

Asthma control test score also showed significant increase in this study, indicating an improvement in the quality of life in our patients. Again, it was more pronounced in the group of patients receiving single allergen immunotherapy.

Patients in both groups A and B showed improvement in asthma severity score. However, there was no statistically significant change in FEV1 after immunotherapy. There was also a marked reduction in medication (SABA) use after 6 months of treatment. Interestingly, 18 patients (60%) stopped their medications completely in group A and 3 (10%) in group B.

These results were also demonstrated in a study which compared single allergen immunotherapy to placebo and they showed a significant decrease in asthma symptom score and medications and increase in the number of well days, nevertheless, there wasn’t significant change in pulmonary function test results,[17] Several other studies have concluded the same results [18, 19, 20, 21, 22], although other studies have demonstrated an improvement in pulmonary function tests in patients receiving immunotherapy [23].

Our results suggest that single allergen immunotherapy was more effective than multiple allergen immunotherapy. The same result was suggested by another study which achieved clinically relevant responses with a US-standardized timothy extract administered in a high dose over a period of 10 months compared to the use of a similar dose of timothy extract combined with 9 other pollen extracts which showed a very limited response [24]. The mild efficacy of multiple allergen immunotherapy has been attributed to mixing of the allergens which causes excessive dilution [25]. We recommend separating allergens into separate vials for better efficacy.
IV. CONCLUSIONS

Although both single and multiple allergen immunotherapy were effective in controlling asthma symptoms and reducing the need for medications, the results of this study suggest that single allergen immunotherapy might be more effective than multiple allergen immunotherapy as evidenced by decrease in serum total IgE but not IL-4 secretion.

References