

# A Study of Immunological and Clinical Effects of Allergen Immunotherapy on Asthmatic Patients in Babylon Province

Dr. Amjed H. A. Al-Mansouy\*

Dr. Naseer J.H. Al-Mukhtar \*

Dr. Mohammed A.K. Al-Sa'adi\*

\* College of Medicine, Babylon University Hilla- Iraq

## Abstract:

**B**ackground: Allergen immunotherapy has important benefits on asthmatic patients by reducing symptoms and medication use and it can be used as an alternative treatment.

**Objectives:** This study aimed to assess the effects of allergen immunotherapy (AI) on asthmatic patients of Babylon Province.

**Materials and methods:** The study was performed on 35 asthmatic patients as immunotherapy group who received allergen immunotherapy and 15 asthmatic patients as control group who remained on pharmacotherapy, the patients were assessed by measurement of peak expiratory flow rate (PEFR), total immunoglobulin E (IgE) and immunoglobulin G (IgG) symptoms and medication use score at 0 time, 3 months, and 6 months.

**Results:** The study showed that 30-40 years age group population were more affected with asthma (44%) and then the incidence decreased with increasing age. Females might be more affected than males (60% compared to 40%). There were significant increase in the mean levels of PEFR and total IgG and significant increase in the mean levels of total IgE. In addition, the study revealed larger reduction in symptoms and medications use score in immunotherapy group in comparison to control group.

**Conclusion:** AI should be used in allergic asthma as one of the modalities of treatment because it can reduce the symptoms and medications use by changing the basic mechanism of asthma.

**Keywords:** Asthma, immunotherapy, PEFR, IgE, IgG, symptoms and medications use score.

## الخلاصة:

**خلفية الدراسة:** هناك فوائد مهمة للقاح المناعي على مرضى الربو وذلك بتقليل الأعراض السريرية واستخدام الأدوية وبالإمكان استخدامه كأحد الطرق البديلة للعلاج .

**الهدف من الدراسة:** استهدفت هذه الدراسة تقييم تأثيرات اللقاح المناعي على مرضى الربو في محافظة بابل. **المواد والطرق:** تمت الدراسة على 35 مريضاً بالربو الذين اخذوا كمجموعة اللقاح (immunotherapy group) وقد تم إعطائهم اللقاح المناعي وتمت مقارنة التأثيرات على 15 مريضاً من الذين اخذوا كمجموعة سيطرة (control group) الذين بقوا على الأدوية المضادة للربو. وتم تقييم التأثيرات التي يسببها اللقاح المناعي وذلك بواسطة قياس المعدل الأقصى لسريان الهواء عن طريق الزفير. قياس الكلوبولينات المناعية IgE and IgG, تسجيل الأعراض السريرية للمرضى، وإدراج الأدوية المستعملة في بداية الدراسة، بعد مرور ثلاثة أشهر، وكذلك بعد مرور ستة أشهر.

**النتائج:** بينت الدراسة أن ازدياد معدلات حدوث مرض الربو في المجموعة العمرية (30-40) سنة أكثر من غيرها إذ بلغت 44% ثم تقل نسبة حدوث المرض بتقدم العمر. بالإضافة الى ازدياد حالات مرض الربو لدى الإناث (60%) أكثر مما هي عليه في الذكور. من ناحية أخرى فإن مستوى peak expiratory flow rate والكلوبولين المناعي (IgG) قد ازداد بشكل

معنوي في مجموعة اللقاح مقارنة بمجموعة السيطرة. وأثبتت الدراسة انخفاض مستوى الكلوبولين المناعي (IgE) بشكل معنوي في مجموعة اللقاح المناعي مقارنة بمجموعة السيطرة. كما بينت الدراسة وجود انخفاض في الأعراض السريرية وكذلك استعمال الأدوية للمرضى في مجموعة اللقاح المناعي أكثر مما هو موجود في المجموعة الضابطة. **الاستنتاج:** أثبتت الدراسة ان اللقاح المناعي يجب استخدامه كأحد طرق علاج مرضى الربو لأنه يعمل على تقليل الأعراض السريرية وكذلك تقليل استعمال الأدوية وذلك بتغيير الميكانيكية الأساسية المسببة لمرض الربو.

## Introduction:

Asthma is a clinical syndrome characterized by chronic reversible airway obstruction, increased bronchial reactivity, and airway inflammation, it affects approximately 155 millions people around the world, although asthma affects people of all ages, it most often starts in childhood, more boys have asthma than girls, but in adulthood, more women have asthma than men (1).

The aetiology of asthma is multifactorial with interaction of both hereditary and environmental factors. Several environmental factors have been implicated like air pollution, hygiene hypothesis, measles infection, and hepatitis A seropositivity (2).

Typical symptoms of asthma include wheeze, shortness of breath, cough, and sensation of chest tightness. These symptoms may occur at any age and may be episodic or persistent. Patients with episodic asthma are usually asymptomatic between exacerbations which may occur during viral respiratory infections or after exposure to allergens (3).

Asthma can usually be diagnosed depending on compatible clinical history and physical examination together with demonstration of variable airflow obstruction by spirometry or peak expiratory flow meter. Chest radiograph is not helpful in diagnosis, but it is performed to exclude other conditions or complications like pneumothorax (4).

Treatment of asthma includes avoidance of trigger factors, use of asthma medications and allergen immunotherapy. These measures can prevent or reduce the frequency and severity of symptoms. The common types of medications include: corticosteroids, bronchodilators, and anti-inflammatory drugs. Allergen immunotherapy is an optimal therapy for many patients and it can reduce the symptoms and the use of drugs (5). Allergen immunotherapy (AI) is also known as hyposensitization or allergy shots. It involves repeated administration of gradually increasing quantities of specific allergens to patients with IgE-mediated

conditions until a dose is reached that is effective in reducing disease severity from natural exposure. It is effective in the treatment of allergic rhinitis, allergic conjunctivitis, stinging-insects (wasp and bee) hypersensitivity, and allergic asthma (6). The clinical indications of AI include: Diagnosis of patients with history of allergy, inability to avoid allergens, inadequacy of drug treatment, side effects of drugs, and desire to avoid long-term medication use (7). It is contraindicated in uncontrolled asthma (including recent asthma exacerbation in the last seven days, current wheezing, and peak expiratory flow below 75% of predicted value), current beta-blockers or angiotensin-converting enzyme (ACE) inhibitors, concurrent significant illness, fever, or fatigue (8).

The aim of the study is to: initiate data base of age and sex of asthma in Babylon Province, evaluation of immunological and clinical effects of AI on asthmatic patients. The immunological effects included measurement of total levels of IgE and IgG before initiation of allergen immunotherapy and after three and six months while the clinical effects included PEFr, evaluation of asthma symptoms and medication use scores before and after the use of immunotherapy, and establishment for the efficacy and the side-effects of allergen immunotherapy use.

## Materials and Methods:

The study was conducted in the Asthma and Allergy Center (AAC) in Babylon Province in the period from October 2007 to May 2008. The study was carried out on chronic stable asthma patients with a history of asthma at least three years. This study included 35 patients (as IT group) and 15 patients (as control group), their ages ranged from 20-60 years and the mean age of patients in the IT group was  $37.3 \pm 1.9$  years, while in the control group it was  $33.6 \pm 2.9$  years. This was consistent with WHO guidelines which recommend 36 patients for immunotherapy (IT) group (9).

From each patient a schedule of follow up consisting of three times after skin testing (zero time, after three months ,and after six months) was made. Those patients were attended to the AAC and were diagnosed by specialized physicians depending on history and clinical criteria. Chest X-rays were taken for all the patients to exclude other diseases. Patients who were smokers, pregnant, severe clinical conditions, unstable asthma, patients on antihistamines, and patients with history or current parasitic infections depending on general stool examinations were excluded.

The control group included patients with asthma not taking allergen immunotherapy (AI) but received pharmacotherapy.

#### Blood samples:

About 5ml of venous blood were withdrawn from each patient and the sera were collected and stored in deep freeze for immunological tests (10).

#### Measurement of peak expiratory flow rate (PEFR):

Peak expiratory flow rate (PEFR) was measured by a device called peak flow meter (PFM). This measures PEFR as liters of air per minute (11).

#### Allergen immunotherapy (allergen vaccine):

##### Method of dilution:

The stock concentration was different for mite and pollen. The stock concentration of mite extract was (1/1000), while for pollen extract was (1/1000) (Allergy vaccine lab., Iraq). Each stock vial contains information including: type of allergen, concentration, volume, storage temperature (2-8 C°), expiration date, company, and state of origin). Each stock of allergen was diluted in multi-vials of phenol according to type of allergen (12). Series of dilutions

for mite and pollen stocks was (1/10,000 ,1/100,000 ,and 1/1000,000) (1/1000, 1/10,000, 1/100,000, and 1/1000,000) respectively (13).

#### Allergy Intradermal Skin Test :

In this test, intradermal injections were done on one of the forearms of each patient. Two sites of injection were recognized by two circles one above the other and marked by pen with two letters to coincide the identity of allergen to be tested. These letters were (M) for mite and (P) for pollen. The sites of injections were cleaned with 70% alcohol ,and intradermal injections were performed with syringes (G 29x1/2). The doses of allergy intradermal skin test were taken from the vial with 1/100,000 dilution and only 10 units were withdrawn by the syringe from each type of allergens (HDM and pollen), after completion of injections, patients were recommended to remain in the AAC for about 15-20 minutes in order to read the results of the test, to monitor for the local reactions, and to manage systemic reactions that may happen occasionally. Obtained results at sites of injection were cleaned with alcohol, also cortisone cream was applied to relieve itching and local reactions (14).

The diameter of the local reactions was measured by a special ruler called phostal-staloral. This ruler consists of circles for measurement of wheal or erythema, each circle represents (5)mm. The skin test in this study was interpreted as recommended by the Huggins and Looney (6) as the following: wheal greater than 5mm or erythema greater than 10mm is considered a positive reaction. Figure (1) shows positive skin test for mite allergen.



Figure (1): Results of allergy skin test. M (mite, positive :erythema and wheal larger than 10mm) and P (pollen, negative: no wheal or erythema).

### Subcutaneous immunotherapy:

The patients included in this study with positive skin tests results were told to come every week to the AAC for a period of three months to complete the build-up phase, then come every month for 3-5 years to complete the maintenance phase (13). The injections were carried out subcutaneously on the outer aspect of the upper arm midway between the shoulder and elbow. The injections were performed according to (7).

#### Doses of injections :

The doses of injections were different according to the phase as follow:

##### A. Build -up phase:

This phase started by the lowest dose which was increased gradually every week. For each diluted vial , four doses were given to each patient, one dose was given weekly, starting from the vial with greatest dilution (1/1000,000).The doses given were calculated by units (U) by syringes which were graduated up to 100 U that equal to one ml. Project of injections

started by 10 U, 20U, then 40 U, and lastly 60 U (15).

##### B. Maintenance phase:

In this phase, patients were given the same dose monthly. This dose represented the largest effective dose that can be tolerated (this means the largest dose that can result in a benefit without causing side effects) and it was different according to the patient response (15).

#### Assessment of patients :

Patients were assessed at the end of the study by three objective measures : PEFr, asthma symptoms and medication use scores. The symptoms and medications use were assessed according to Bousquet score (16).The total level of IgE was measured as recommended by Monobind company (USA),while the total level of IgG was measured according to LTA company (Italy) recommendations.

#### Statistical Analysis :

Statistical analysis was performed using the SPSS programme. Analysis of variance (ANOVA) was used to evaluate changes over time between groups. P values of less than or equal to 0.05 were considered to indicate statistical significance (17).

#### Results:

Table (1) showed the ages distribution of the patients' groups.

Table(1): Age of asthmatic patients and control groups

Group	Number	Age (years) mean±SEM
Immunotherapy (IT)	35	37.35±1.9
Control (patients)	15	33.6±2.9

SEM: Standard error of mean (p >0.05).

The ages of patients ranged from 20-60 years. There was no significant difference in ages between the studied groups (p >0.05). The ages range of the patient was ( 20-60) years and this was in agreement with most studies about AI like Creticos et al 1996 (18).

Table (2) showed the distribution of all asthmatic patients (50 patients)

according to age group. Age group 30-40 years old was the dominant (P 0.05) which included highest number of asthmatic patients with (22) out of (50). Females were more affected than males.

Table (4-2): Distribution of all asthmatic patients according to age groups

Age group	Number	Percentage
20-30	13	26%
31-40	22	44%
41-50	9	18%
51-60	6	12%
Total	50	100%

The patients were sensitive to HDM more than pollen. Some patients were sensitive to both HDM and pollen. The percentage of patients who were sensitive to HDM was 70%, HDM and pollen was 20%, and pollen was 10% as shown in table (3).

Table (3) : Percentage of allergens sensitivity

Type of allergen	Percentage of sensitive patients
House dust mite	70%
House dust mite +pollen	20%
Pollen	10%

The levels of PEFr were significantly increased in the immunotherapy group in comparison to the control group ( $p < 0.05$ ), they were also significantly increased within the same immunotherapy group at the different times of follow-up (figure 2).

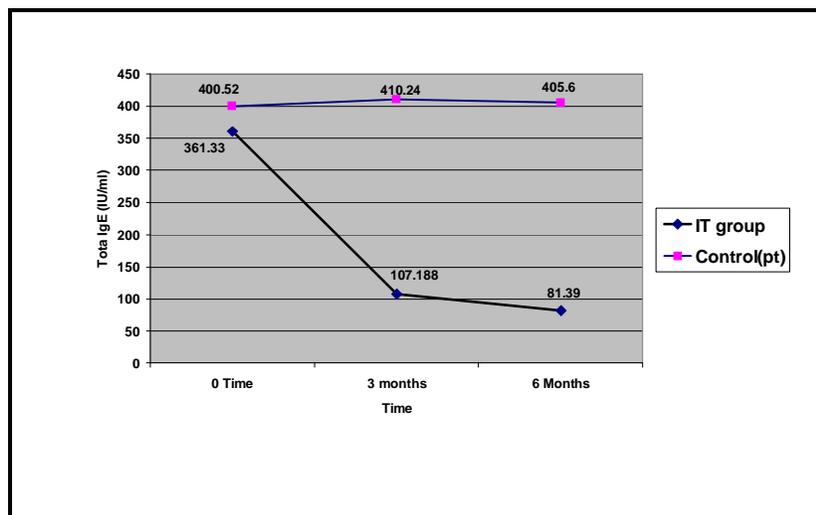


Figure (2) : The levels of total immunoglobulin E in immunotherapy and control (patients) groups at three times(0 time, after 3 months, and after 6 months) (p 0.05).

The mean levels of total IgE were significantly decreased after immunotherapy in immunotherapy group in comparison to control group (P 0.05). In addition, the

mean /levels were significantly decreased within the same immunotherapy group at the three times of follow-up as shown in figure (3).

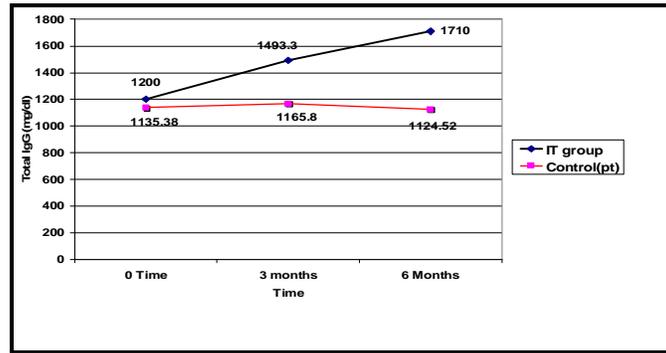


Figure (3) : The levels of total immunoglobulin G in immunotherapy (IT) and control (patients) groups at three times(0 time, after 3 months, and after 6 months) (p 0.05).

while the mean levels of total IgG were significantly increased (p 0.05), were significantly increased within the same immunotherapy group at the three times of follow-up (figure 4).

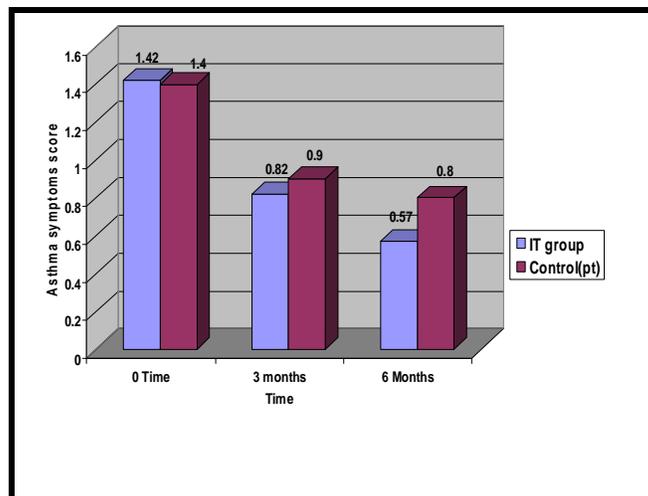


Figure (4): Asthma symptoms score in immunotherapy (IT) and control (patients) group at 0 time, after 3 months, and after 6 months (p>0.05).

Figure (5) showed the mean asthma score in the immunotherapy (IT) and control groups at 0 time, 3 months, and 6 months. Bousquet score (2001) (16) was depended in this study for assessment of symptoms. Both groups showed a continuous reduction in

symptoms scores over the period of the study.

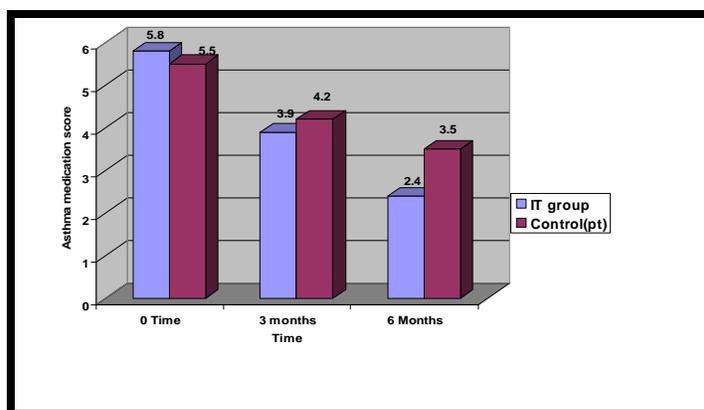


Figure (5) : Asthma medications score in immunotherapy (IT) and control (patients) group at 0 time, after 3 months, and after 6 months ( $p > 0.05$ ).

The results of scores of medications use according to the time were apparent in figure (6) which showed the mean asthma medications in the IT and control groups at 0 time, 3 months, and 6 months. Both groups showed a

continuous reduction in medication use over the period of the study.

The study showed reduction in symptoms scores in patients taking immunotherapy more than patients taking pharmacotherapy, also more reduction in medication scores in the immunotherapy group than the control group and this means more reduction in use of drugs in the immunotherapy group but the difference was nonsignificant between the immunotherapy and control groups.

Side-effects were recorded according to Bousquet score (2001) (16). The mean level of side-effects score was 0.3, of the 35 asthmatic patients who underwent AI, only 2(5%) patients had generalized urticaria, 3 (9%) patients had local reactions larger than 3 cm and these reactions were treated with antihistamines, all the remaining 30 (86%) patients had local reactions less than 3cm and resolved spontaneously as shown in table (5). The table also showed that no severe side-effects were reported. The mean score of side-effects was  $0.3 \pm 0.2$  as shown in table (5). Immunotherapy was

tolerated by most patients and the incidence of side-effects was low.

No severe systemic side-effects were reported, only two patients had generalized urticaria and were treated well with anti-histamines. The reactions occurred within 20-30 minutes following injections.

### Discussion:

The ages range of patients was 20-60 years and it was selected because the wheal and flare are not clear beyond these ages, moreover the level of IgE is usually stable and constant at this age group. The sex difference may be due to female sex hormones because about 40% of women report a premenstrual increase in asthma symptoms and also they have a regulatory role in  $\beta_2$ -adrenergic function, abnormal regulation may be a possible mechanism for premenstrual asthma (19).

PEFR was a very important indicator in the assessment of severity of asthma that provided objective assessment of the severity of asthma. At the beginning of the study, the mean levels in both groups were nearly similar (450 vs. 454 L/min), but they were 10 L/min higher in immunotherapy group than control group after three months while 35L/min higher after six months.

The observed increase in IgG had led to the concept that this immunoglobulin act as a blocking antibody. Circulating IgG may block access of the allergen to mast cells, or it may bind to the mast cells and through recruitment of inhibitory IgE receptors inhibit the mast cell response (20;21). Till et al 2004 (22) pointed that allergen immunotherapy was associated with a marked increase in blocking IgG antibodies and decrease in allergen specific IgE concentrations that occurs due to immune deviation from  $T_{H2}$  response with dominant production of IL-4 and IL-5 toward  $T_{H1}$  response with production of interferon gamma (IN- ) and IL-2 and this deviation occurs because of induction of a subset of T-regulatory cells with allergen specific increase in the production of IL-10 and transforming growth factor beta (TGF- ).

The study also showed reduction in symptoms and medication use score in both groups with a larger reduction in immunotherapy group but it was nonsignificant and this might be due to the short period of the study although many studies also lasted for six months and some with significant results while others with nonsignificant results.

Thus we conclude that allergen immunotherapy should be strongly considered for patients with poor asthma control or adverse reactions to medications. From the results expressed above we recommend that physicians can consider allergen immunotherapy in addition to appropriate pharmacotherapy when asthma control remains inadequate and using of another immunizing route that cause clonal deletion or elicit  $T_{H1}$  than  $T_{H2}$  response.

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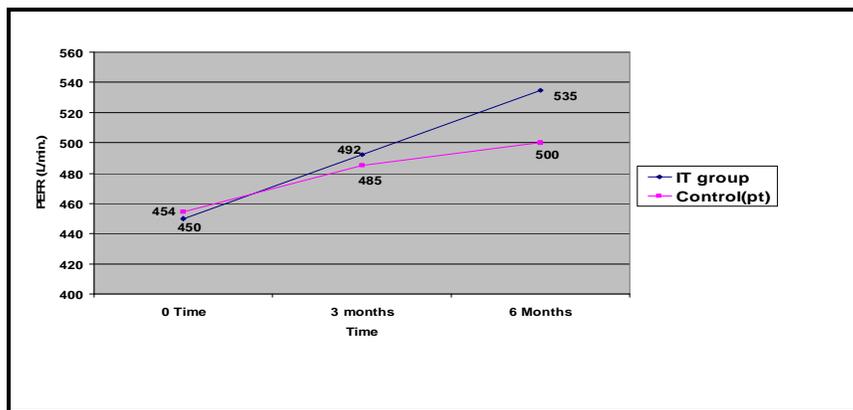


Figure (1) : The levels

of peak expiratory flow rate in immunotherapy and control (patients) groups at three times (0 time, after 3 months, and after 6 months) (p < 0.05).

Table (4) : Side-effects reaction score of allergen immunotherapy in immunotherapy group

Reaction	score	No. of patients	Final score	Meanscore
Local				
Enduration and/or erythema < 3 cm	0	30	0	0.3
Enduration and /or erythema >3 cm	1	3	3	
Systemic				

Generalized urticaria	2	2	4	
Generalized pruritus and sneezing, nasal congestion	3	0	0	
Wheezing, tachypnea, and decrease of peak expiratory flow rate	4	0	0	
Anaphylaxis, hypotension, severe wheezing, and laryngeal edema	5	0	0	
Cardiopulmonary arrest	6	0	0	
Total	21	35	7	