



A Study of Some Physiological and Immunological Changes of Asthmatic Patients in Babylon Province

A Thesis

**Submitted To The Council Of The College Of Medicine
To The Babylon University**

**In Partial Fulfillment Of The Requirement For The Master Degree In
Medical Physiology**

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2008 A.D.

1429 A.H.

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دراسة لبعض التغيرات الفسلجية والمناعية عند مرضى

الربو في محافظة بابل

رسالة مقدمة

إلى مجلس كلية الطب/جامعة بابل كجزء من متطلبات نيل درجة

الماجستير في الفسلجة الطبية

من قبل

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1429 هـ

2008 م

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا غَشَّيْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صدق الله العلي العظيم
(سورة البقرة: الآية 32)

الخلاصة

استهدفت هذه الدراسة وصف بعض العوامل المسببة لمرض الربو في محافظة بابل، وتقييم بعض الخواص الفسلجية وعلاقتها بمرض الربو، وكذلك تقييم تأثيرات اللقاح المناعي على مرضى الربو. تضمنت الخواص الفسلجية مجاميع الدم (blood groups) وقياس خضاب الدم (hemoglobin)، وكذلك معرفة مستوى مقياس الاس الهيدروجيني للدم (blood pH) لدى مرضى الربو.

تضمن اللقاح المناعي تعريض كل مريض الى جرعة متزايدة تدريجياً تستهدف تقليل الاستجابة المناعية والالتهابية وبالتالي تقليل أعراض الحساسية عنده، وتكون اللقاح المناعي الذي يشمل مرحلتين هما: مرحلة البناء و تتضمن هذه المرحلة إعطاء المريض جرعة واحدة اسبوعياً لمدة ثلاثة أشهر تتبعها مرحلة الادامة عن طريق إعطاء المريض جرعة واحدة شهرياً تستمر لمدة 3-5 سنوات. تم تقييم تأثيرات اللقاح في بداية الدراسة، بعد مرور ثلاثة أشهر، وكذلك بعد مرور ستة أشهر على 35 مريضاً بالربو من الذين يمتلكون استجابة موجبة في الاختبار الجلدي لحشرة العث (house dust mite) وحبوب الطلع (pollen) وتمت مقارنة التأثيرات على 15 مريضاً من الذين اخذوا كمجموعة سيطرة (control group) ولم يستلموا اللقاح المناعي لكن بقوا على الأدوية المضادة للربو.

وتم تقييم التأثيرات التي يسببها اللقاح المناعي وذلك بواسطة تسجيل الأعراض السريرية للمرضى، وادراج الأدوية المستعملة، قياس النسبة المطلقة لخلايا الدم الحمضية (absolute eosinophil count)، قياس المعدل الأقصى لسريان الهواء عن طريق الزفير، وقياس الكلوبولينات المناعية IgE and IgG.

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, وهو يحدث في المدن أكثر من الأرياف. وكانت نسبة المرضى الذين يملكون تاريخ عائلي موجب لمرض الربو 40%, أما نسبة صنف الدم (O) في المرضى فكانت أكثر من غيرها, وكانت نسبة المرضى المصابين بمرض الاسترجاع المريئي المعوي هي 52% وكان مستوى (pH) الدم لمرضى الربو 7.7 بينما كان 7.36 للأشخاص الأصحاء, وكان مستوى خضاب الدم (Hb) 13.2 للمرضى و 12.6 للأشخاص الأصحاء.

أما نسبة الحساسية لحشرة العث (house dust mite) وحبوب الطلع والحساسية المزدوجة (حشرة العث وحبوب الطلع) فقد بلغت 70%, 10%, و20% من الحالات على التوالي.

وأثبتت الدراسة انعدام الفرق المعنوي في نسبة خلايا الدم الحمضية بين مجموعة اللقاح (immunotherapy group) و مجموعة السيطرة (control group). من ناحية أخرى, فإن مستوى peak expiratory flow rate قد ازداد بشكل معنوي في مجموعة اللقاح مقارنة بمجموعة السيطرة.

وأثبتت الدراسة انخفاض مستوى الكلوبولين المناعي (IgE) وكذلك زيادة مستوى الكلوبولين المناعي (IgG) بشكل معنوي في مجموعة اللقاح المناعي مقارنة بمجموعة السيطرة.

كما بينت الدراسة وجود انخفاض في الأعراض السريرية للمرضى في مجموعة اللقاح المناعي أكثر مما هو موجود في المجموعة الضابطة, وحصول انخفاض كبير في استعمال الأدوية في المجموعة الأولى أكثر منه في المجموعة الثانية, كما بينت الدراسة انخفاضاً كبيراً في حساسية الجلد للمواد المسببة للحساسية (allergens) و ظهر ذلك بصورة واضحة في الاختبار الذي اجري في نهاية الدراسة.

We , the examiner committee , certify that we have read the thesis entitled (A Study of Some Physiological and Immunological Changes of Asthmatic Patients in Babylon Province) and have examined the student (Amjed Hassan Abbas Al-Mansoury) in its contents, and that in our opinion it is accepted as a thesis for the degree of Master of Science in Medical Physiology with excellent estimation.

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Summary

This study aimed to describe some of the risk factors of asthma in Babylon Province in Iraq; evaluate some of the physiological parameters in relation to asthma and assess the effects of allergen immunotherapy (AI) on asthmatic patients of Babylon Province. The physiological parameters included: blood groups, measurement of hemoglobin and blood pH.

Allergen immunotherapy involved exposing a patient to gradually increasing doses of specific allergens with the intention of decreasing allergic and inflammatory response, ultimately leading to a sustained decrease in allergic symptoms. A build-up phase (once weekly injections for three months) was followed by a maintenance phase (once monthly injections) that generally continued for 3-5 years.

The effects of allergen immunotherapy were assessed at 0 time, 3 months, and 6 months on 35 asthmatic patients with positive skin test to house dust mite and pollen (as immunotherapy group) and the effects were compared to 15 asthmatic patients as control group who remained on pharmacotherapy.

The changes caused by AI were assessed by symptoms and medication use scores, measurement of absolute eosinophil count, peak expiratory flow rate (PEFR), and measurement of total immunoglobulin E (IgE) and immunoglobulin G (IgG).

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 were more affected than males (60% compared to 40%). There were 62% of patients came from urban areas in comparison to 38% of cases who came from rural areas. The percentage of patients with positive family history was 40% of the cases.

Patients with blood group O were more affected with asthma. Gastro-esophageal reflux disease was found in 52%. Blood pH value was 7.7 in asthmatic patients while it was 7.36 in healthy subjects. Blood hemoglobin was 13.2g/dl in asthmatic patients compared to 12.6 gm/dl in healthy subjects.

The study also showed that the percentage of sensitivity to house dust mite, pollen, and mixed sensitivity (house dust mite pollen) were 70% , 10%, and 20% from cases respectively.

There was no significant difference in the level of absolute eosinophil count between the immunotherapy (IT) and control groups. In addition, no change was found at the three times of patients assessments. The level of peak expiratory flow rate was significantly increased in the IT group as compared to the control group ($p \leq 0.05$). The level of total IgE was significantly decreased in the IT group as compared to the control group. Also, there was a significant difference in the of total IgG between the IT and control group ($p \leq 0.05$). In addition, the study revealed marked reduction in symptoms and medications use score in the IT group with the progression of treatment while

less reduction in the control group. The study also demonstrated that the IT was a safe procedure. The mean score of side-effects was 0.3.

Introduction:

Asthma is a clinical syndrome characterized by chronic reversible airway obstruction, increased bronchial reactivity, and airway inflammation. Asthma 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. It affects all races but African American are more likely than Caucasians to be hospitalized for asthma attacks and die from asthma (Cengizler & Misirlioglu, 2006).

The aetiology of asthma is multifactorial with interaction of both hereditary and environmental factors. Genetic factors alone cannot account for the significant increase in prevalence. Several environmental factors have been implicated like air pollution, hygiene hypothesis, measles infection, and hepatitis A seropositivity (Dale & Federman, 2003).

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 (Hauser *et al.*, 2005).

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. In more difficult situations, where the above tests are negative, an exercise test, a histamine or methacholine bronchial provocation test or a trial of oral corticosteroids (eg, prednisolone 30 mg daily for 2 weeks) may be required. An elevated sputum or peripheral blood eosinophil count or increased serum level of total IgE may also be useful. Chest radiograph is not helpful in diagnosis , but it is performed to exclude other conditions or complications like pneumothorax (Boon *et al.*,2006).

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 which reduce swelling and inflammations in the airways, bronchodilators which open the airways by relaxing the muscles in the airways, and anti-inflammatory drugs which reduce inflammation and allergic response. Allergen immunotherapy is an optimal therapy for many patients and it can reduce the symptoms and the use of drugs (Douglas & O’Hehir,2006).

Aims of the study:

This study aims to:

1. Initiate data base of age, sex and residential distribution of asthma in Babylon Province.
2. Study the distribution of family history among asthmatic patients.
3. Study the relation of some physiological features (blood pH, blood hemoglobin, eosinophils count, and blood groups) to asthma.
4. Study the relation between gastro-esophageal reflux disease (GERD) and asthma.
5. Evaluation of IgE and IgG levels before initiation of allergen immunotherapy and after three and six months.
6. Evaluation of asthma symptoms and medication use scores before and after the use of immunotherapy.
7. Establishment for the efficacy and the side-effects of allergen immunotherapy use.

Abbreviations:

Symbol	Description
AAC	Asthma and allergy centre
ACE	Angiotensin-converting enzyme
AEC	Absolute eosinophil count
AI	Allergen immunotherapy
AII	Allergen injection immunotherapy
APCs	Antigen-presenting cells
BCG	Calmette-Guerin bacilli
CO ₂	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CDC	Centre of disease control
EAR	Early allergic response
ERV	Expiratory reserve volume
FEV ₁	Forced expiratory volume in first second
FVC	Forced vital capacity
FRC	Functional residual capacity
GERD	Gastro-esophageal reflux disease
GC	Glucocorticoid
Hb	Hemoglobin
HDM	House dust mite
HSA	Human serum albumin
IC	Inspiratory capacity
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	interleukin
IN- γ	Interferon gamma
IRV	Inspiratory reserve volume
IT	Immunotherapy

K ₂ -EDTA	Dipotassium ethylene diamine tetra-acetic acid
LBW	Low birth weight
O ₂	Oxygen
Po	Pollen
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
PFM	Peak flow meter
PFT	Pulmonary function test
SCIT	Subcutaneous immunotherapy
SD	Standard deviation
SE	Standard error
Th ₁	T helper type ₁
Th ₂	T helper type ₂
TLC	Total lung capacity
TGF-β	Transforming growth factor beta
TV	Tidal volume
WBCs	White blood cells
WHO	World health organization

ACKNOWLEDGEMENTS

I would like to express my thanks to Allah the Most Gracious and Most Merciful.

It is a pleasure to express my deep appreciation to my supervisors Assist.Prof. Dr.Naseer Jawad Al-Mukhatar and Assist.Prof. Dr. Mohammed Abed Kadhim Al-Sa'adi for their valuable guidance during the progress of the present work.

I am grateful to Dr.Safaa Al-Turaihy and Dr.Salih Al-Mukhatar for their help in accomplishing this study.

I am extremely thankful to Dr. *Abdul-Kareem J. Al-Bermany*, head of department of physiology and medical physics, college of medicine.

I am deeply indebted to Dr.Ali Khair Allah, dean of college of medicine, Babylon University for providing all the medical facilities.

I would like to thank the medical staff in asthma and allergy centre in Babylon Province.

We certify that this thesis was prepared under our supervision at the Department of Medical Physiology, College of Medicine, University of Babylon and hereby recommend to be accepted in partial fulfillment of the requirements for the degree of M.Sc. in medical physiology...

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Recommendations of the Head of Department of Physiology and Medical Physics:-

According to the available recommendations, I forward this thesis for debate by the examining committee.

Assist.Prof.Dr. Abdul-Kareem J. Al-Bermany

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Conclusions:

1. Significant higher incidence of asthma in adults occurred in 30-40 years age group.
2. Asthma can affect both sexes with non significant females elevation.
3. Non significant higher incidence of asthma occurred in urban areas of Babylon Province more than rural areas.
4. Non significant increases of blood pH and blood group O were associated with mild to moderate asthma.
5. Non significant larger reduction of asthma symptoms and medication use was found in the immunotherapy group in comparison to the control group.
6. Peak expiratory flow rate (PEFR) was significantly increased in the immunotherapy group compared to the control group.
7. The total immunoglobulin E (IgE) was significantly decreased while the total immunoglobulin G (IgG) was significantly increased in the immunotherapy group compared to the control group.
8. Peripheral blood eosinophils count remained unchanged in both groups.
9. Administration of allergen immunotherapy appeared to be safe, although there was a potential for systemic reaction to occur.
10. Allergen immunotherapy should be strongly considered for patients with poor asthma control or adverse reactions to medications.

Dedication

To

My Father, Mother ,Brother and Sisters

My wife,

My sons (Waeel and Ali)

5.1.Distribution of ages:

Table (4-1) showed the mean ages in the immunotherapy and control groups. There was no significant difference in ages between the groups. The ages range of the patient was (20-60) years (figure 4-1) and this was in agreement with most studies about AI like Creticos *et al.*,(1996). This ages range 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. Before the age of 20 years , the level of IgE begins to rise from early childhood to reach adult level by 5 to 7 years of age and between the ages of 10-14 years, the level may be higher than those in adults. After age 60 years, IgE level may decline slightly and be lower than the levels observed in adults (Winter *et al.*,2000).The study showed that asthma was more common in 30-40 age groups and then the incidence decreased with increasing age (table 4-2), so the results of distribution of age in this study was in accordance to Pawar,(2002).

5.2.Sex distribution:

Females were more affected than males as shown in figure (4-1).The percentage of females was 60%, whereas that of male was 40% and this agrees with study of Maddox and Schwartz, (2002) which stated that women aged 40 years have greater prevalence of asthma of the same age group. Also, Mingomataj *et al.*, (2008) found that the percentage of females was (62.6 %) while that of male was (37.4 %).This 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 β 2-adrenergic function, abnormal regulation may be a possible mechanism for premenstrual asthma (Tan *et al.*,1997).

5.3.Family history and asthma:

The study showed that the percentage of patients with family history of asthma was 40% (figure 4-2). Similar result was reported by Cengizlier and Misirlioglu, (2006).

All of the recent asthma family history studies confirmed that asthma was strongly associated with family history of asthma. Fuhlbrigge, (2001) mentioned that about three-fourths of all asthma cases were hereditary, if a person has a parent with asthma, he or she is three to six times more likely to develop asthma than someone who does not have a parent with asthma. Weiss, (2001) reported that asthma in an offspring is more likely if one parent has asthma with a risk of about 1 in 5, and even more likely if both parents have asthma with a risk of about 2 out of 3, also he mentioned that identical twins were more likely than non-identical twins to show concordant asthma.

Mingomataj *et al.*, (2008) found that the percentage of family history was 23.3%. Eseverri *et al.*, (1998) found out that among the risk factors of asthma, 69% of the patients had a family history.

5.4.Residential distribution :

According to their residence, the distribution of asthmatic patients in this study revealed that asthma was more prevalent in urban than rural areas as shown in figure (4-3).Similar result was also produced by Kilpelainen *et*

al., (2000) who found out that the farm environment reduce risk of asthma in young adults with or without family history of atopy, besides people who lived in urban areas are in continuous and direct exposure to air pollution on a daily basis and were more likely to develop asthma. Eseverri *et al.*, (1998) studied the risk factors for development of asthma and among these risk factors, he found that all of the patients were from urban and urban-industrial areas.

Rural residence was identified as the strongest protective factor against asthma in epidemiological studies and was likely associated with environmental exposure such as life style, diet, and hygiene (Nicolou *et al.*, 2005). Numerous epidemiological studies had provided evidence that migration from countries of low asthma prevalence (nonindustrial countries to those of high asthma prevalence (industrial countries) was associated with increased risk of asthma (Solé *et al.*, 2007).

Sensitization to pollen was higher in urban areas than rural areas despite pollen counts are higher in rural areas and this can be interpreted either as due to development of immunological tolerance to pollen allergens in rural areas or due to adjuvant effects of urban pollution. Riedles *et al.*, (2000) found out that exposure to stables and/or farm milk is protective only if prior to one year of age and so if the mother had been exposed to one or both of these factors during pregnancy, also it could be due to a variety of factors including breast feeding, non-immunization, and exposure to fermented milk products. Studies in animals have shown that air pollutants such as SO₂, NO₂, and O₃ increase the permeability of the mucus membranes of the airway tract, favouring the development of allergic reactions (Nicolai, 2002). Kauffman *et al.*, (2002) found

out that IgE levels are significantly lower in those who permanently live in the country and in particular in those who live for $>$ or $=$ 10 years, in addition, positive skin prick tests (SPT) were significantly less prevalent in those who permanently lived in the country. Majeed *et al.*, (2008) found out that most of the asthmatic children lived in the urban areas of Hyderabad.

5.5.Asthma status and gastro-esophageal reflux disease (GERD):

The frequency of GERD in asthmatic patients was high and increased proportionately with severity of asthma. In the present study, significant and positive correlation was observed between the asthma episode and GER, that may reach 52% of total asthmatic immunotherapeutic and control patients groups (table 4-3). These results were in a good agreement with data reported by Plottova *et al.*, (2001) who found out that GERD present in 45-89% of asthmatic patients.

Kiljander *et al.*, (1999) reported that 53% of asthmatic patients had pathological GERD. In addition to investigation of Jedynac-Wasowicz *et al.*, (2007) who observed that acidic gastro-esophageal reflux was diagnosed in (56%) asthmatic children. Most of those patients (88%) came from urban areas. Carmona-Sanchez *et al.*, (1999) reported in a study on 50 patients with asthma that 27 (54%) reported heartburn and regurgitation at least twice a week, the pH-recording showed pathologic GER in 13 patients with mild asthma (57%), in 13 with moderate asthma (81%) and in all patients with severe asthma (100%).

5.6. Effect of asthma on blood pH value :

The mean level of blood pH in patients with asthma was 7.7 and it was 7.36 in healthy subjects (table 4-4). In asthmatic patients the level was more alkaline than normal individuals and this agreed with Hauser *et al.*, (2005) who revealed that most individuals with asthma have hypocapnea and respiratory alkalosis. Similar result was reported by Chaulier *et al.*, (2007). Ferri, (2003) found out hypocapnea and increased pH in patients with mild asthma. Seema and Wasserman, (2005) mentioned that in early stages of asthma, there was respiratory alkalosis and hypocapnea as the tachypneic patient expires carbon dioxide, with worsening airflow obstruction, the blood pH becomes increasingly acidic due to retention of carbon dioxide. The physiology of these changes was explained by the patient response to airflow obstruction. Initially, tachypnea (hyperventilation) can compensate for the hypercapnea that results from obstruction. However, a combination of worsening pulmonary obstruction and respiratory fatigue from prolonged accessory muscles use will eventually compromise ventilation, this results in increase in PaCO₂ with respiratory acidosis and these changes should quickly alert the clinician for impending respiratory failure.

This study was different from another study performed by Lissac, (2007) who mentioned that the change in blood pH occurs only in severe asthma which lead to metabolic acidosis.

5.7. Hemoglobin distribution:

There was no significant difference in the level of hemoglobin (Hb) between asthmatic patients and healthy subjects. In asthmatic patients, the mean

level was 13.2 g/dl while in healthy group the mean level was 12.6 g/dl as shown in table (4-5). This agreed with Dale and Federman, (2003) who revealed that asthma has no effect on blood Hb

5.8. Asthma and ABO erythrocytes surface antigens :

Good knowledge about ABO blood group had expanded to include diverse and numerous arrays of antigenic determinants on erythrocytes. Approximately 600 erythrocytes antigens are known, each antigen is controlled by one gene (Chen *et al.*, 2005).

The results of ABO blood group and their distribution among asthmatic patients showed that patients with blood group (O) were more susceptible than the other types of ABO blood groups (figure 4-4). These data were in a good agreement with the results of Khetsuriani and Gamkrelidze, (1995). Also, similar observation was reported by Chen *et al.*, (2005) and Al-Shamma *et al.*, (2008) who found out that blood group (O) phenotype individuals were significantly associated with asthma ($P \leq 0.03$) and may act as one of the predominant factors for environmental triggers of allergy for asthmatic children in Taiwan. Moreover, they found out that the risk for the development of severe bronchial asthma was higher in patients with blood type B, whereas in blood type O a mild to moderate degree of severity of was more characteristic and this gives a support to this current study because patients that were selected were those with mild to moderate asthma.

Ronchetti *et al.*, (2001) revealed that the proportion of blood group (O) in asthmatic children was higher than in controls, thus confirming the association

found in adults. Kauffman *et al.*, (1996) reported that significantly lower lung function and higher prevalences of wheezing and asthma were observed in subjects of blood group O.

This study showed that the lowest percentage of blood groups in asthmatic patients was blood group AB and this agreed with Dzhvarisheishvili and Ksenofontov, (1981) who mentioned that in respiratory allergy, the group at greatest risk was this who had type O blood., whereas resistance to respiratory allergies was associated with blood type AB.

5.10.Absolute eosinophil counts (AECs) :

The levels of absolute eosinophils count were not changed by immunotherapy and there were no significant differences between the immunotherapy and control groups as shown in table (4-6). This result was in accordance to a study by Wang *et al.*,(2006) . The current study also showed that the levels of AECs in asthmatic patients are within normal ranges. This might be due to the patients that are selected for this study had mild to moderate asthma and the level of eosinophils counts in peripheral blood is usually related to severity of asthma as revealed by many studies like (Rebuck, 1987; Griffin *et al.*, 1991; Seema & Wasserman, 2005).

Besides, Bruijnzeel, (1989) mentioned that patients with asthma may have a normal number of eosinophils in their blood suggesting that asthma may be related to a high level of total IgE but not to blood eosinophil counts. Moreover, Hospers *et al.*,(2000) mentioned that peripheral blood eosinophilia was associated with increased mortality of asthmatic patients because increased

number of peripheral blood eosinophils may reflect an increased inflammatory response, resulting in tissue injury.

5.9. Peak expiratory flow rate (PEFR) :

A very important indicator in the assessment of severity of asthma was the measurement of PEFR 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 as shown in figure (4-5). The study revealed that there were significant differences in the level of PEFR between the two groups ($p < 0.05$). Similar result was reported by others (Adkinson *et al.*, 1997; Wang *et al.*, 2006; Lue *et al.*, 2007). Ross *et al.*, (2000) found out significant improvement in pulmonary function. Olsen *et al.*, (1997) found out, in a study on thirty-one adult patients with asthma caused by house-dust mites, significant increase in PEFR in the immunotherapy group after 1 year of the study.

5.11. Distribution of allergens types:

The majority of patients were sensitized to house dust mite (HDM) as shown in table (4-6). This was in accordance to (Allen-Ramey *et al.*, 2005; de Builderling *et al.*, 2008) who reported that the majority of patients were sensitive to HDM. Mingomataj *et al.*, (2008) found out that HDM sensitization was 84.4% while pollen sensitization was 16.7%. Sarpong and Karrison, (1998) found that the percentage of HDM sensitization was 55%. In a study by Joshi *et al.*, (2003), percentage of house dust mite (HDM) was 82.67%. Koshak *et al.*, (2007) studied the prevalence of skin reactivity to indoor allergens and

revealed that the highest reactivity was to HDM (87%).In addition, they reported that the increase in asthma prevalence was due to the increased exposure to these allergens.

5.12. Evaluation of total immunoglobulin E (IgE) and immunoglobulin G (IgG):

The study showed that the mean levels of total immunoglobulin E (IgE) were significantly decreased in the immunotherapy group with the increasing time of immunotherapy in comparison to the control group ($p \leq 0.05$) (figure 4-6), while the mean levels of total immunoglobulin G (IgG) were significantly increased ($p \leq 0.05$) (figure 4-7). Similar results were revealed by many other studies (Gleich *et al.*, 1982; Ohashi *et al.*, 1997; Ebner *et al.*, 1999; Akdis & Akdis, 2007). Rossi *et al.*, (2007) and Francis *et al.*, (2008) reported that after subcutaneous immunotherapy (SCIT) all patients had high titres of serum IgG antibodies. Lue *et al.*, (2006) also found out after six months of immunotherapy significant decrease in the level of total IgE and significant increase in the level of IgG.

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 (Gehlhar *et al.*, 1999; Wedi & Kapp, 2004). Till *et al.*, (2004) 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 Th2 response with dominant production of IL-4 and

IL-5 toward Th1 response with production of interferon gamma (IN- γ) 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- β). IN- γ and IL-2 cause downregulation of IgE response and avert antibody production toward IgG production. These events are accompanied by suppression of late allergic response in the skin and lung and long term disease suppression which is apparent following discontinuation of immunotherapy. From the results of concentrations of IgE and IgG, we conclude that isotype switching but not clonal deletion was induced in the IT group. This means that Th2 response still induced than Th1 response.

5.13.Symptoms and medication score:

The study showed reduction in symptoms scores in patients taking immunotherapy more than patients taking pharmacotherapy (figure 4-8), also more reduction in medication scores in the immunotherapy group than the control group (4-9) and this means more reduction in use of drugs in the immunotherapy group but the difference was nonsignificant between the immunotherapy and control groups. 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. This result was similar to a study by Pham-Thi *et al.*, (2007) who illustrated a large reduction of asthma symptoms and decreased use of inhaled corticosteroids and inhaled β 2-agonist without significant difference between groups. Other studies with similar results were (Sheikh, 1996; Altintas *et al.*, 1999; Lue *et al.*, 2006).

Other studies reported significant improvement in symptoms and medications requirement. Ross *et al.*, (2000) found out significant improvement in symptoms and decrease in medication requirement. In addition to significant clinical manifestation improvement had been demonstrated by Mathew & James, (2007), this improvement was associated with a reduced need for anti-inflammatory and bronchodilator treatment. At same time, Ferrer & García-Sellés, (2003) confirmed that symptoms and medication scores also showed a significant response after 3 and 6 months of treatment only in immunotherapy group.

Cevit *et al.*, (2007) revealed that patients in the immunotherapy group experienced significant improvement in asthma symptoms ($P \leq 0.001$) and medication scores ($P < 0.001$) whereas control group did not. Wang *et al.*, (2006) found out the mean symptom scores in the active group showed a significant lower scores than the control group till the end of the study. There were significant differences in subjective judgments of the improvement about overall asthmatic symptoms, exacerbation severity and exacerbation frequency between the active group and the control group. The trend line of medication scores showed a significant decline in active group, whereas the control group did not show a significant difference. Francis *et al.*, (2008) found out that grass pollen immunotherapy was effective in reducing overall symptom scores ($P < 0.05$).

Ameal *et al.*, (2005) found out the immunotherapy group has a median reduction of 91.5% in symptom scores, whereas the placebo group decreased by 86%, also the medication scores decreased in both groups (56% in the

immunotherapy group and 11.4% in the placebo group). García-Robaina *et al.*, (2006) revealed the median improvement in the immunotherapy group over placebo was 53.76% in total symptom and 58.09% in medication scores.

5.14.Skin test reactivity :

The study revealed marked reduction in skin test reactivity. This agreed with Altintas *et al.*,1999; Joshi *et al.*,2003; Ibero & Castillo, 2006). In addition, Cevit *et al.*, (2007) found out that unlike the control group, the immunotherapy group showed a tendency for skin reactivity to mite to decrease. Similarly, Wang *et al.*,(2006) revealed that Skin test response decreased in actively treated subjects only. Varney *et al.*,(2003) reported 36% reduction in skin test sensitivity to *D. pteronyssinus* in the immunotherapy group, while the placebo group values were unchanged.

5.15.Side-effects score:

The mean score of side-effects was 0.3 ± 0.2 as shown in table (4-8). 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. Similar studies were reported by others (Pawar,2002; Ferrer & García-Sellés,2003; Rossi *et al.*,2007). Wang *et al.*,2006 reported that the frequency of systemic adverse reactions was 5.7% of the total injections in the immunotherapy group, all adverse reactions were mild or moderate. The majority occurred within 30 minute after injection and were controlled well

with symptomatic therapy. No any severe systemic adverse reaction was shown in the study. Similar results were reported by Lue *et al.*,(2006). Altntas *et al.*, (1999) reported that the mean of the immunotherapy side-effect score was 0.5 ± 1.3 . Ameal *et al.*, (2005) revealed that mild to moderate systemic effects (rhinitis, mild bronchospasm, and urticaria) occurred in one out of 1500 injections, there was one severe (near-fatal) anaphylaxis per million injections, and one death per 2.5 million injections, AI might cause one death in Australia every 8 years. On the basis of data from a large survey, the risk of a fatal anaphylactic reaction from AIT had been calculated to be 1 in 2.5 million injections (about 3 deaths per year in the United States); near-fatal reactions occurred once in every 200,000 injections (Amin *et al.*,2006).

In another study , only 0.5% of allergic injections resulted in a systemic reaction (Matloff *et al.*, 1993).

Most severe adverse events result from giving an incorrect dose, giving the wrong extract, or giving the vaccine to a patient who has unstable asthma or is taking β -blockers . As adverse reactions are not predictable, safety can be enhanced by adhering strictly to the requirement that patients remain in the clinic for a minimum of 30 minutes (ideally, 45 minutes) after each injection (even maintenance doses). This facilitates early access to medical treatment if an adverse reaction occurs (Bousquet & Michel, 1994;Weiner,2006).

Concentrated extracts must be diluted with an appropriate sterile diluent prior to first use on a patient for treatment or intradermal testing. All

concentrates of allergenic extracts are manufactured to ensure high potency and therefore have the ability to cause serious local and systemic reactions (Ameal *et al.*,2005).

Systemic reactions may occur if the patient receive more extract than can be tolerated (overdosage).This may occur because of an error in the volume of extract injected, an incorrect dilution used, or the patient may be exposed to airborne or environmental antigens simultaneously with injection of the same antigens (Amin *et al.*,2006).

5.16.Efficacy of allergen immunotherapy in general (overall evaluation):

Generally, the results of this study revealed significant difference between IT and control groups in the following parameters: PEFr, total IgE, and total IgG. The study also showed great improvement in symptoms score, medication use, and skin sensitivity test that did not reach the degree of significance.

Reha & Ebru, (2007) found out allergen specific immunotherapy was the only specific and curative approach in the treatment of asthma. The study revealed that with the use of probability value (P value) , the results of PEFr, IgE, IgG, symptoms and medications score were significant indicating a significant difference between the two groups.

Walker *et al.*, (2001) reported reduction in medications and symptoms scores with no significant local or systemic side effects. Mital *et al.*,(2003) revealed, in a study composed of 76 patients with perennial asthma, significant symptomatic relief with continuous treatment of specific immunotherapy for 6 months, complete to slight relief of symptoms was observed in 86.84% while 13.16% had no relief.

Saraclar *et al.*, (1998) found out at the 12th month of specific immunotherapy, total asthma symptom scores, bronchodilators requirement, and immediate skin reactivity to HDM were decreased, while PEFr increased to the pretreatment values ($p < 0.05$ for each). These parameters remain unchanged in the control group. Dahl *et al.*, (2008) studied the efficacy and safety of allergen immunotherapy over 2 years and reported that there was highly significant reduction in asthma symptoms and medications scores, clinical improvements were paralleled by significant changes in the levels of total immunoglobulins (IgE and IgG), the treatment was well tolerated and adverse events led to withdrawal occurred in less than 1% of participants, there were no serious adverse events related to treatment.

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3. Materials & Methods

3.1. Materials:

3.1.1. Equipment and Apparatuses:

The equipment and apparatuses used in this study were shown in table (3-1):

Table (3-1) Equipment and Apparatuses

Equipment	Company	Origin
Light microscope	Olympus	Japan
Micropipette (1000 μ L)	Oxford	USA
Centrifuge	PLC series	USA
Peak flow meter	Medicare	India
Incubator	Memmert	Germany
Freezer	Concord	Lebanon
Refrigerator	Concord	Lebanon
Microscope slides	Sail Brand	China
Microscope cover glass	Ataco	China
Automated differential counter	Erma	Japan

Phostal-staloral	Stallergenes	France
Universal pH indicator	Macherey-Nagel	Germany
Sahli apparatus	Ataco	China
Ocular lens	PLC series	USA
Syringes (29G×½)	Micro-fine Plus	USA
Syringes (23G×1)	Proton	Malaysia.

3.1.2.Chemicals:

The chemical materials that were used were shown in table (3-2) :

Table(3-2): Chemicals

Chemical	Company	Origin
Dipotassium ethylenediamine tetra-acetic acid(k ₂ -EDTA)	Afma-Dispo	Jordan
Glacial acetic acid	Afma-Dispo	Jordan
HCL	BDH	England
70% alcohol	Fluka chemika	Switzerland
Leishman stain	Crescent	Saudi Arabia

3.1.3.Immunological Materials :

Table(3-3) shows the immunological materials as well as mite and pollen extract.

Immunological materials	Company	Origin
IgE kit	Monobind	USA
IgG Plate	LTA	Italy
Mite extract	Allergy vaccines lab.	Iraq
Pollen extract	Allergy vaccines lab.	Iraq
Phenol	Allergy vaccines lab.	Iraq

3.2.Patients :

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. This was consistent with WHO guidelines which recommend 36 patients for

immunotherapy (IT) group (WHO,1998).This also agreed with many studies. Some of these studies were summarized in the table (3-3) :

Table (3-3).Some studies about allergen immunotherapy

Author (Reference)	Age (years)	No. of patients (IT/control)
Saraclar <i>etal</i> (1998)	15-65	13/5
Durham <i>etal</i> (1999)	16-70	37/14
Altntas <i>etal</i> (1999)	4-8	34/5
Pawar <i>etal</i> (2002)	10-70	36/15
Lue <i>etal</i> (2006)	6-12	20/9
Wang <i>etal</i> (2006)	6-45	29/10
Cevit <i>etal</i> (2007)	6-16	19/12
Jarzynska <i>etal</i> (2008)	20-50	32/10

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, in addition it included 10 healthy subjects without any signs and symptoms of asthma.

Table (3-4):Number of individuals in the studied groups

Group	Number
Immunotherapy group (IT)	35
Control (patients) group	15
Control (healthy) group	10

3.3.Methods :

3.3.1.Blood Samples:

Venous blood samples were obtained from antecubital vein by means of disposable plastic syringes with wide bore needles (23G x1) , the skin was cleaned with 70% alcohol and 5 ml of blood were withdrawn , 2ml of this blood were put into dipotassium ethylenediamine tetra-acetic acid(K₂-EDTA) tube and the blood mixed well to prevent clotting of the blood , the other 3 ml were delivered into disposable sterile plain tubes. At the same time , one drop of EDTA blood tubes was taken and put on glass slides for blood films preparation , also 0.02 ml of this blood had been taken and then mixed with 0.4 ml of glacial acetic acid for the determination of the total white blood cells counts ,in addition to 0.2 ml taken for hemoglobin measurement (Dacie & Lewis,2005).

The blood in the plain tubes was allowed to clot , then the serum was separated by centrifugation for 15 minutes at 3000 round per minutes (RPM) and the serum obtained was put in another disposable sterile plain tubes and stored in deep freeze for biochemical and immunological tests.

3.3.2.Physiological methods:

3.3.2.1.Measurement of peak expiratory flow(PEF):

Peak expiratory flow (PEF) was measured by a device called peak flow meter(PFM) (figure 3-1).This measures PEF as liters of air per minute.

3.3.2.1.1.Steps of measurements (Miller,2004):

- 1.Patient should be standing up.
2. Peak flow meter (PFM) was held horizontally with the indicator faces upward and on zero level.
- 3.Patient should take deep breath as possible as he can.
- 4.Patient should place the lips tightly around the mouthpiece and blow as hard and fast as he can.
- 5.The score on the meter was checked.
- 6.These steps were repeated for three times for each examined subject.
- 7.The highest score (in 6) was recorded.



Figure (3-1): Peak flow meter.

3.3.2.2.Measurement of blood pH :

EDTA blood tubes were left for about one hour to obtain plasma then pH paper was immersed in the plasma for getting a color on the stick paper which was then compared to the control standard colors (Macherey-Nagel, Germany).

3.3.2.3.Measurement of blood hemoglobin concentration by Sahli's acid hematin method:

Principle: Hemoglobin (Hb) is converted into acid hematin by hydrochloric acid (HCL).The brown color of the resulting compound is matched against a brown glass standard in a comparator.

Procedure:

The hemoglobin tube was filled with HCL to the mark 20 by means of a dropper. About 0.2 ml of blood was put in the same tube and mixed well with HCL by means of glass rod, then left for 10 minutes. In this interval at least 95% of the color of acid hematin was developed. Then the solution of acid hematin was diluted by adding distilled water, drop by drop, stirring the mixture after each addition of distilled water and comparing the color of the solution to the color of the standard , this dilution continued till the color of the solution matches perfectly with that of the standard. At this moment the hemoglobin concentration was read in grams percent by reading the bottom of the meniscus (Dacie & Lewis,2005).

3.3.2.4.Determination of total and differential white blood cells (WBCs) (Dacie and Lewis,2005):

3.3.2.4.1.Determination of total WBCs count:

About 0.02 ml of the blood had been taken from the anticoagulant tube and mixed with 0.4 ml of glacial acetic acid ,then one drop was taken and placed on a Neubauer 's chamber at the space between the cover slip and the chamber then the WBC were counted under the microscope (x 40).The cells were counted in each of the four corner secondary squares on both sides of the chamber. The cells were counted starting in the upper left corner square, then the right hand square, drop down to the next row and the counting continued from the right square to the left square. The WBCs were calculated in cubic mm on the basis of the number of cells counted, area and the dilution made.

$$\begin{aligned}
 \text{Cells/cubic mm} &= \frac{\text{cells counted} \times \text{blood dilution} \times \text{chamber depth}}{\text{Area of chamber counted}} \\
 &= \frac{\text{cells counted} \times 20 \times 10}{4} \\
 &= \text{cells counted} \times 50
 \end{aligned}$$

3.3.2.4.2.Determination of differential WBCs count :

From previous anticoagulant tubes, one drop of blood was taken and smeared to make blood film and then left to dry. These smears were stained with Leishman's stain for five minutes then flooded with distal water for ten minutes and washed with distal water also and left to dry again and examined under the microscope (with oil immersion).The area where morphology of the cells is clearly visible was chosen. The differential

counting was done by moving the slide in area including the centre and periphery of the smear. A total of 100 cells was recorded by using the automated cell counter, then the percentage of eosinophils was calculated. The calculation of absolute eosinophils count was done by multiplying the number of total WBCs counted by the percentage of eosinophils.

3.3.2.5.General stool examination :

This examination was carried out by placing one drop of normal saline over a glass slide then small amount of stool was taken by wooden stick, mixed and covered by cover slide and examined under the (x40) microscope (Dacie & Lewis,2005).This test was carried out to check for the presence or absence of parasitic infections. All patients with parasitic infections were excluded.

3.3.3.Immunological methods:

3.3.3.1.Allergen immunotherapy(allergen vaccine):

3.3.3.1.1.Method of dilution:

The vials of allergens, mite and pollen, and phenol (diluent) were brought to the center in cool boxes, one box for each type. The stock concentration was different for mite and pollen as shown in the tables (3-4a and 3-4b) which illustrated the methods of dilution for HDM and pollen respectively. The stock concentration of mite extract was (1/1000),while for pollen extract was (1/100). Each stock vial contains informations including: type of allergen, concentration, volume, storage temperature (2-8 C°),expiration date, company, and state of origin). The volume of mite extract was (6) ml while volume of pollen extract was (3.4) ml. Each stock

of allergen was diluted in multi-vials of phenol according to type of allergen (Francis *et al.*,2008). Generally half ml was taken from the allergen stock vial and put in one vial of phenol which contains (4.5) ml to obtain (5) ml diluted vial, then half ml was withdrawn from this diluted vial and put in another vial of phenol which also contains (4.5) ml to obtain another (5) ml diluted vial and these steps were serially repeated till reaching the largest dilution which was (1/1000,000). Series of dilutions for mite stock was 1/10,000 ,1/100,000 ,and 1/1000,000 ,so three diluted vials were obtained for each half ml taken from mite stock vial. Series of dilution for pollen stock was 1/1000, 1/10,000, 1/100,000, and 1/1000,000 so for each half ml taken from pollen vial , four diluted vials were obtained. All diluted vials (stock and diluted) were stored in the refrigerator at 2-8 C° (Weiner,2006) .

Table(3-4a) Dilution of house dust mite showing method of dilution of half ml of house dust mite to obtain three diluted vials with progressive decrease in concentration

Dilution	Extract	Diluent /ml	Volume/ ml	Concentration
0	stock	0	6	1/1000
1	0.5 ml stock	4.5 ml	5	1/10,000
2	0.5 ml diluent 1	4.5 ml	5	1/100,000
3	0.5 ml diluent 2	4.5 ml	5	1/1000,000

Table(3-4b) Dilution of pollen showing method of dilution of half ml of pollen to obtain four diluted vials with progressive decrease in concentration

Dilution	Extract	Diluent /ml	Volume/ ml	Concentration
0	stock	0	3.4	1/100
1	0.5 ml stock	4.5 ml	5	1/1000
2	0.5 ml diluent 1	4.5 ml	5	1/10,000
3	0.5 ml diluent 2	4.5 ml	5	1/100,000
4	0.5 ml diluent 3	4.5 ml	5	1/1000,000

3.3.3.1.2.Allergy Intradermal Skin Test :

3.3.3.1.2.a.Procedure:

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 as in figure (3-2).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 Asthma and Allergy Center 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 (Joshi,2000).



Figure (3-2): Mite allergen skin sensitivity test (intradermal injections).

3.3.3.1.2.b.Interpretation of skin test results:

The diameter of the local reactions was measured by a special ruler called phostal-staloral (figure 3-3).This ruler consists of circles for measurement of wheal or erythema, each circle represents 5mm. The skin test in this study was interpreted as recommended by the Huggins and Looney (2004) as the following: wheal greater than 5mm or erythema greater than 10mm is considered a positive reaction. Figure (3-4) shows positive skin test for mite allergen.



Figure (3-3): The special ruler for measurement of erythema or wheal (each circle represents 5mm, the circles and triangles at the edge represent the distances between sites of allergen injections in children and adults respectively).



Figure (3-4): Results of allergy skin test. M (mite, positive :erythema and wheal larger than 10mm) and P (pollen, negative: no wheal or erythema).

3.3.3.1.3.Subcutaneous immunotherapy:

The patients included in this study with positive skin tests results were told to come every week to the Asthma and Allergy Centre 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 (Weiner,2006).

3.3.3.1.3.a.Site of injection:

The injections were carried out subcutaneously on the outer aspect of the upper arm midway between the shoulder and elbow as shown in figure (3-5).The site was cleaned with alcohol and before injecting the vaccine, the plunger of the syringe (G29x1/2) was withdrawn back to avoid intravenous injections. Mild pressure was applied at the site after injections (Mathew and James,2007).

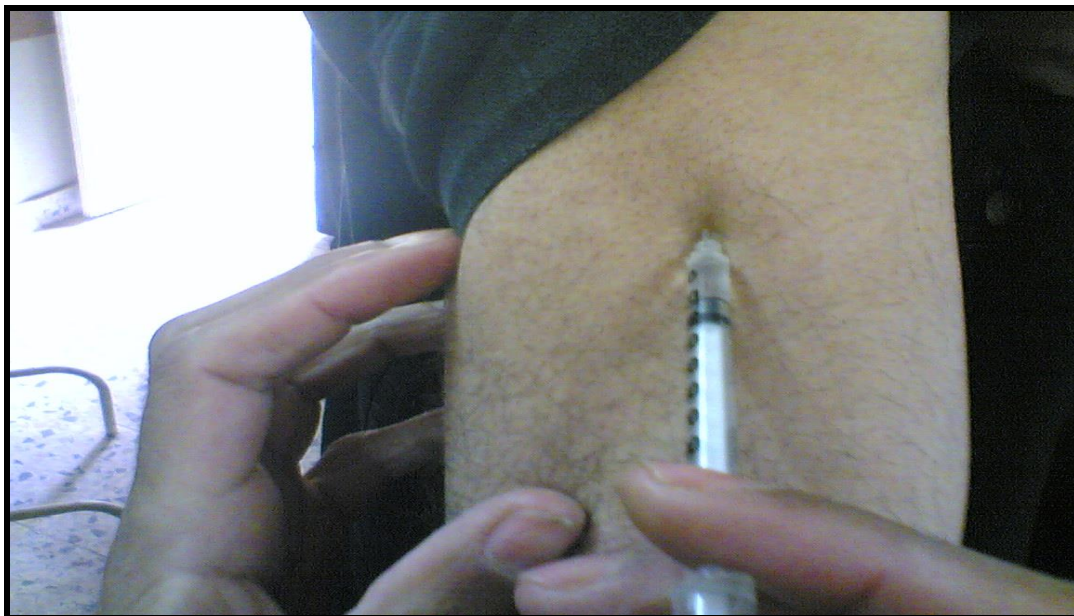


Figure (3-5): Subcutaneous immunotherapy.

3.3.3.1.3.b.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 (Pham-Thi *et al.*,2007).

B. Maintenance phase:

In this phase, patients were given the same dose monthly. This dose represented the largest effective dose that can be tolerated and it was different according to the patient response (Pham-Thi *et al.*,2007).

3.3.3.1.4.Assessment of patients :

Patients were assessed at the end of the study by three objective measures :early skin response, asthma symptoms and medication use scores and PEF. Immediate skin response was assessed by intradermal injection of HDM and/or pollen according to the sensitivity of patients. Immediate skin response occurred within 15 minutes after injection. The response was expressed as the size of erythema or wheal after injection (Mathew and James,2007).

The symptoms and medications use were assessed according to Bousquet score (2001) as shown in table (3-5) and (3-6) respectively.

Table (3-5) Asthma symptoms score (Bousquet, 2001)

Symptom	Score
None	0
Rhonchi at expiration	1
Rhonchi at inspiration and expiration	2
High pitched wheezing at rest	3

Table(3-6) Asthma medication score (Bousquet, 2001)

Medication	Score
No medication	0
Inhaled salbutamol	1
Oral ketotifen	2
Inhaled steroid	3
Inhaled steroid + oral aminophylline	4
Oral steroid <20 mg	5
Oral steroid >20 mg	6

3.3.3.1.5. Assessment of side-effects:

Side-effects were recorded according to reaction score scale (Bousquet, 2001) as shown in table (3-7).

Table (3-7) Reaction score scale (Bousquet, 2001)

Reaction	Score
Local	
Enduration and/or erythema < 3cm	0
Enduration and /or erythema >3cm	1
Systemic	
Generalized urticaria	2
Generalizd pruritus and sneezing, nasal congestion	3
Wheezing ,tachypnea, decrease of peak expiratory flow rate	4
Anaphylaxis, hypotention, severe wheezing, and laryngeal edema	5
Cardiopulmonary arrest	6

3.3.3.2. Enzyme immunoassay for the quantitative determination of immunoglobulin E (IgE) concentration in human serum:

Principle :

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native

antigens. In this procedure, the immobilization takes place during the assay at the surface of microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody. Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody forming an Antibody-Antigen complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an Enzyme labeled Antibody-Antigen-Biotinylated Antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained (Monobind, USA).

Procedure :

The microplates' wells for each serum reference, control and patient specimen were formatted in duplicate, about 25 μ l of the appropriate serum reference, control or specimen was put into the assigned well, then 100 μ l of the IgE Biotin reagent was added to each well and it was very important to dispense all reagents close to the bottom of the coated well, the microplate

was swirled for 20-30 seconds for mixing and covering, then incubation for 30 minutes at room temperatures. The contents of the microplate were discarded by decantation by tapping and plotting the plate dry with adsorbent paper, then 3000 μ l of wash buffer was added, this step was repeated two additional times for a total of three washes, after that 100 μ l of the Anti-IgE-HRP labeled antibody was added to each well, the microplate was gently swirled for 20-30 seconds for mixing and covering, incubation for 30 minutes at room temperatures. The contents of the microplate were discarded by decantation, then 300 μ l of wash buffer was added, then decantation and this step was repeated two additional times, 100 μ l of the working substrate was added to all wells, incubation for 15 minutes at room temperatures, 50 μ l of stop solution was added to each well, then the adsorbance in each well was read at 450 nm. (Monobind, USA).

3.3.3.3.Determination of the immunoglobulin G (IgG) protein by single radial immunodiffusion plate :

Principle :

The examined protein, diffusing in agarose gel containing a specific antibody will form an immuno-complex, visible as a ring around the well. The ring diameter is proportional to the concentration of the analysed protein. The proportion corresponds to the diffusion time. In fact, at the end (48h), the square of diameter will be in linear proportion to the concentration. However a reference table is provided showing the relation between any concentration and the end of procedure (LTA, Italy).

Procedure :

The plate was removed from its envelope and left to stand at room temperature for few minutes so that any condensed water in the wells can evaporate. The wells were filled with 5 μ l of samples and controls and waited it has been completely adsorbing before handling the plate. The plate was closed and placed in a moist chamber, then waiting for 48 hours.

Results interpretation:

The diameters of the precipitating rings were measured after 48 hours and compared to their corresponding concentrations on reference table (LTA, Italy).

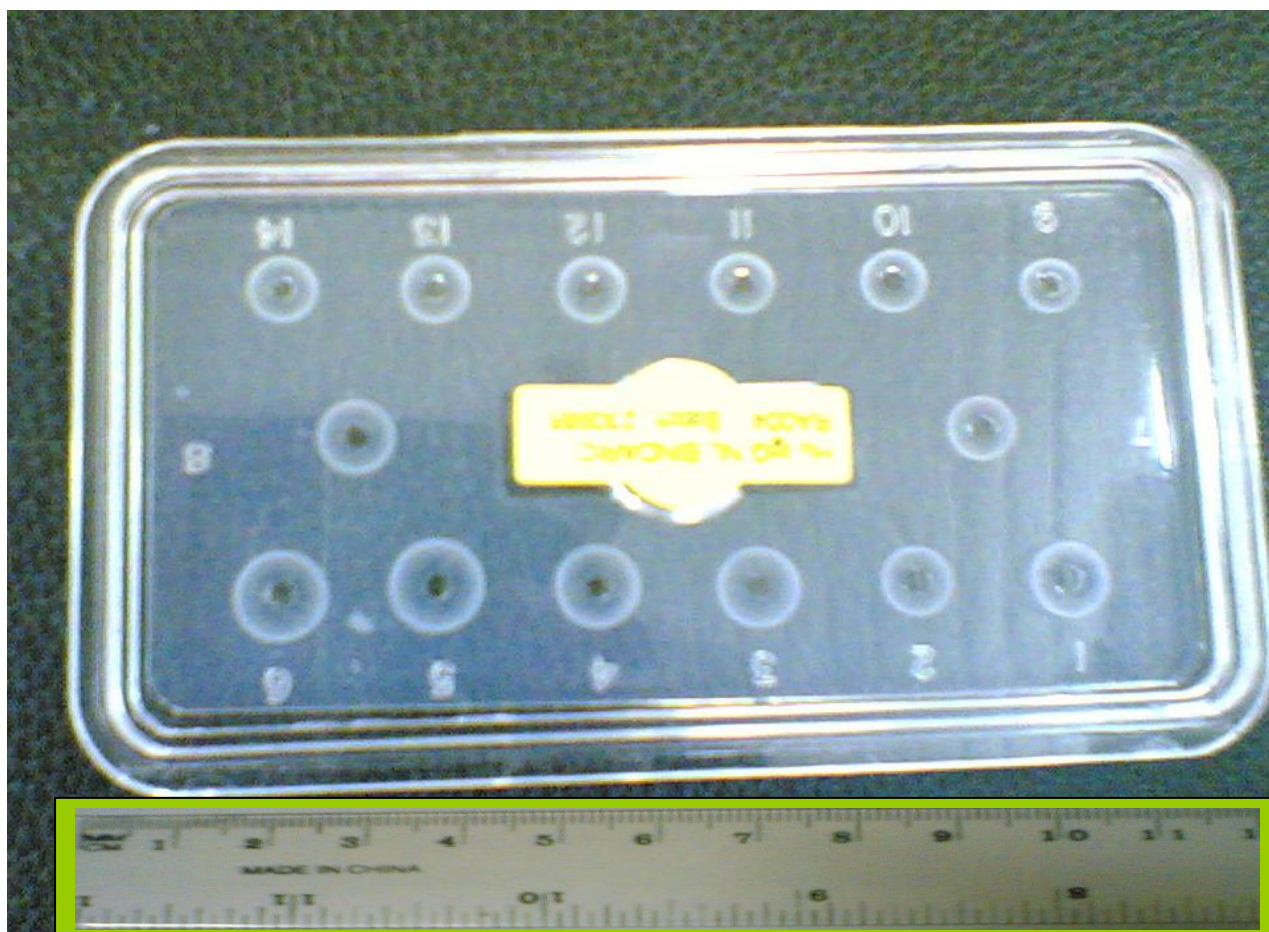


Figure (3-6) : Results of single radial immunodiffusion for immunoglobulin G.

3.4.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, while p values larger than 0.05 indicated statistical nonsignificance. T test was also used for some physiological parameters to compare between IT and control groups (Daniel, 1999).

Recommendations:

- 1.Allergen immunotherapy should be used on a routine basis in patients sensitive to house dust mite and pollen.
- 2.Allergen immunotherapy should be administered only in hospitals or in specialized centers to counteract adverse reactions.
- 3.Patients should be kept under close supervision for the first 30 minutes after each injection and any severe or generalized reactions must be recorded in order to modify the dose.
- 4.Allergen immunotherapy should not be recommended in severe, unstable and nonallergic asthma.
- 5.Physicians can consider allergen immunotherapy in addition to appropriate pharmacotherapy when asthma control remains inadequate.
- 6.Using of another immunizing route that cause clonal deletion or elicit Th1 than Th2 response.

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2.1.The Respiratory System :

The respiratory system supplies the body with oxygen (O_2) and gets rid of carbon dioxide (CO_2). The respiratory tract is anatomically divided into upper and lower respiratory tract. The upper respiratory tract consists of the parts outside the chest cavity: nose, pharynx, larynx, and the upper trachea. Whereas, The lower respiratory tract consists of parts found within the chest cavity: the lower trachea and the lungs which include :bronchi, bronchioles, alveolar ducts and alveoli (figure 2-1). Other parts of the respiratory system are the pleural membranes and the respiratory muscles that form the chest cavity: the diaphragm and the intercostals muscles (Scanlon & Sanders,2007).

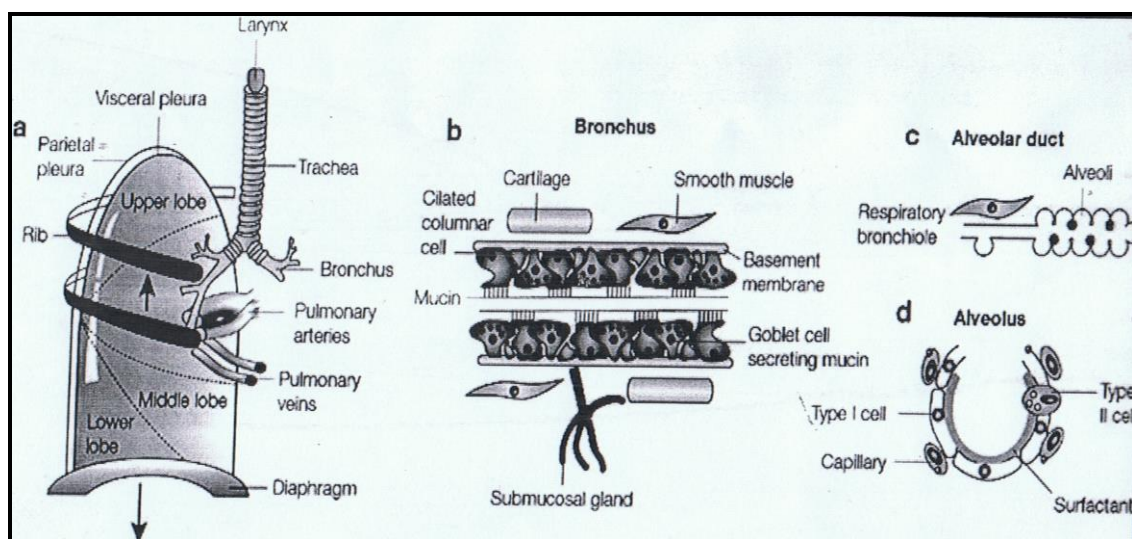


Figure (2-1): Schematic diagram of lung anatomy with cross-sections of bronchi, bronchioles, alveolar ducts, and alveoli.a: Gross anatomy of lung and thorax.b: Microscopic anatomy of bronchial wall.c: View of terminal airway and alveoli.d: Alveolar structure (Effros,2006).

The structure and the function of the respiratory system especially the lungs represent a remarkable compromise and adaptation that allow it to serve a number of very different needs; such as gas exchange, filtration of blood flowing to the systemic circulation, and metabolism of metabolically active peptides in the circulation, as well as, ability of respiratory system to withstand environmental attacks by innumerable particles, droplets, acids, bases, organisms and many other foreign materials over the lifespan of human being (Effros,2006).

The lungs are soft, spongy, cone-shaped organs located on each side of the chest. They are separated from each other by the mediastinum (ie, the space between the lungs) and its contents which include the heart, blood vessels, lymph nodes, nerve fibers, thymus glands, and esophagus. The top or apex of each lung extends into the lower part of the neck, just above the first rib, whereas the base of the lung lies against the diaphragm. The lungs are divided into lobes: three in the right lung and two in the left lung (Aw *et al.*,2001). Each lung is enclosed by thin double-layered serous membrane called the pleura. The outer parietal layer lies against the chest wall while the inner visceral layer lies against the lungs. The intrapleural pressure is always negative in relation to the alveolar pressure which helps to prevent lung collapse because of its elastic recoil. During inspiration, contraction of the diaphragm and intercostals muscles causes the thoracic cage to be elevated leading to expansion of the lungs and the intrapleural pressure becomes more negative than during expiration so the air will be sucked in from the atmosphere into the lungs. The process of ventilation can

be divided into three parts: ventilation where air passes between the atmosphere and the respiratory portion of the lungs, perfusion where the blood flows through the respiratory tissues, and diffusion where gas exchange occurs between the alveoli and the blood (Porth,2007).

2.2.Embryological Development of the Respiratory System

The respiratory system does not carry out its physiological function (of gas exchange) until after birth. Development of respiratory system and diaphragm occurs early in embryonic life. Respiratory system is an outgrowth of the ventral wall of the foregut. The epithelium of the larynx, trachea, bronchi, and alveoli originates in the duodenum. The muscular, cartilaginous, and connective tissue components arise in the mesoderm (Effros, 2006).

The bud of the lung develops into two main bronchi : the right part is composed of three secondary bronchi and three lobes; the left part is composed of two secondary bronchi and two lobes (Ganong, 2005 ; Sadler, 2006).

During the seventh month of embryo, gas exchange between the blood and air in the primitive alveoli is possible. During embryo developmental stages ,the alveoli are filled with fluid with little protein, mucus and surfactant layer which is produced by type 2 alveolar epithelial cells and its function is to form a phospholipids material coat on the alveolar membranes. As the respiration begins , the lung fluid is resorbed except for the surfactant layer, which prevents the collapse of the alveoli during expiration by reducing the surface tension at the air-blood capillary interface (Saddler ,2006).

At the last two weeks of gestation period alveolar formation commences and at the time of birth, several million alveolar-like structures are present. Alveoli continue to develop following birth, until the child reaches about 8-10 years of age. In all, a total of about 300 millions alveoli are formed with a total surface area of about 70m² (Massaro & Massaro, 2002; Dinana, 2008)..

2.3.Pulmonary Function Test (PFT) :

The pulmonary function tests are used for detecting the nature and severity of pulmonary disorders and to measure the response to therapy. These functions are usually based on measurements of lung volumes and maximal flow. However, the pulmonary function tests are compared to normal values that are estimated on the basis of the height, age, sex, and race of the patient (Marion *et al.*, 2001; Fulambark *et al.*, 2004; Effors, 2006). The normal quiet breathing is about 15 complete respiratory cycles per minute. The lung and the air passages are never empty and, as the exchange of gases takes place only across the walls of the alveolar ducts and alveoli, the remaining capacity of the respiratory passages is called the anatomical dead space (about 150 ml) (Guyton & Hall, 2006).

2.3.1.Pulmonary Volumes and Capacities (Porth, 2007) :

Pulmonary volumes and capacities are used in diagnosis and monitoring of respiratory diseases. All pulmonary volumes and capacities are about 20-25% less in women than men and they are greater in large and athletic people than in small and asthenic people.

2.3.1.1.Tidal volume(TV): Is the volume of air which passes into and out of the lungs (inspired or expired) during each cycle of quiet breathing. It is about (500ml) in the average adult young man.

2.3.1.2.Inspiratory reserve volume(IRV) : Is the extra volume of air that can be inspired to the lungs during maximal inspiration and the normal IRV of adult man is about 2500-3000 ml.

2.3.1.3.Expiratory reserve volume(ERV) : Is the extra amount of air that can be expired from the lungs during maximal forceful expiration after the end of normal tidal expiration, and it is about 1500 ml in normal adult man.

2.3.1.4.Residual volume(RV) :Is the volume of air remaining in the lungs after the most forceful expiration and it equals about 1500 ml.

2.3.1.5.Inspiratory capacity (IC) : Is the amount of air that can be inspired with maximum effort. It consists of tidal volume plus the inspiratory reserve volume. Normal IC in the adult man is about 3500 ml.

2.3.1.6.Functional residual capacity (FRC) : Is the amount of air remaining in the air passages and alveoli at the end of quiet expiration.(The FRC consists of RV plus the ERV, and it is about 3000ml).

2.3.1.7.Vital capacity (VC) : Is the maximum volume of air which can be moved into and out of the lungs with each breath. The $VC=IRV+TV+ERV$. The VC is about 4.5 L in the normal adult man.

2.3.1.8.Total lung capacity(TLC) : Is the maximum volume of air in the lungs after a maximal inspiratory effort. TLC is determined by the

strength contraction of the inspiratory muscles and the inward elastic recoil of the lungs and the chest wall. TLC consists of the RV,TV, IRV, and ERV.TLC in the healthy adult man is about 6 litres. (TLC=Vital capacity+ residual volume=5800 ml).

2.3.2.Peak Expiratory Flow Meter (PEFM) :

Peak expiratory flow meter(PEFM) is a small, hand-held device used for evaluation of asthma by monitoring changes in airflow through the bronchi and thus the degree of obstruction in the airways (Nunn and Gregg, 1989). It measures the maximum ability of expiration, so the readings are higher when patients are well, and lower when the airways are constricted. From changes in recorded values, doctors and experienced staff can determine lung function, severity of asthma symptoms, and treatment options (Oga,2001). The measurement of PEF was pioneered by Wright who produced the first meter in the late 1950s (Essig,2008).The PEFM measures the flow during the 10 milliseconds of forced expiration and records it as PEF directly on a dial. In healthy subjects, PEF is about (600) L/min. for men and (450) L/min. for women at the age of 20 years , falling to about (450) and (300) L/min. respectively in the eighth decade of life. Individual variation of these mean values may be up to (100) L/min. (Miller,2004).

2.3.3.Spirometry :

Spirometry is a simple important test that can be carried out at the hospital or the doctor's clinic. It is useful in the initial evaluation of patients suffering from shortness of breath, who may have one of

respiratory or cardiac disorders (Fuhlbrigge, 2001). It is performed by a device called a spirometer. It measures forced expiratory volume in first second (FEV1) and forced vital capacity (FVC) and this is given in terms of percentage, FEV1/FVC, which can be compared against charts that give the normal reading for a person's age, sex, and height. In normal subjects, the percentage is more than 75 (Marion *et al.*, 2001).

2.4.Asthma :

2.4.1. Historical Background :

The word "asthma" is derived from the Greek "aazein" which means sharp breath. The word first appeared in Homer's Iliad (Tobin, 2001). Hippocrates was the first who used it in reference to the medical condition in 1450 BC. He thought that the spasms associated with asthma were more likely to occur in tailors, anglers, and metal workers. Galen, after six centuries, wrote much about asthma, noting that it was caused by partial or complete bronchial obstruction. In the 17th century, Ramazzini noted a connection between asthma and organic dust (Pinnock & Shah, 2007). The use of bronchodilators started in 1901 and in the 1960s the inflammatory components of asthma were recognized and the anti-inflammatory medications were added to the regimens. (Eseverri *et al.*, 1998).

2.4.2.Epidemiology :

. Asthma is a common disease which affects both adults and children. It continues to cause significant mortality (Kitch & Levy,

2000). It is currently a worldwide problem with increasing prevalence in both children and adults. Total prevalence is estimated to be 7.2% of the world's population and it affects about 155 millions people around the world. Asthma is the most common chronic illness and is the highest chronic condition causing hospitalization in children (Dale & Federman, 2003). Prevalence varies in different countries and even within different areas of the same country. In 1998, asthma affects about 17.3 million persons in the united states, representing a 27% increase over 1993. Globally, asthma is responsible for around 180,000 dead cases annually (Tobin, 2001).

2.4.3. Risk factors of asthma :

2.4.3.1. Age:

Disease onset can occur in persons at any age, but it usually starts during childhood and it represents the most common chronic disease of childhood, many people outgrow asthma especially boys then it regains in the 20s and 30s (Porth, 2007).

2.4.3.2. Sex :

In children, the prevalence of asthma is higher in boys than girls. Male to female ratio is 3:2 in children ages 6-11 and increases to 8:5 ratio in those ages 12-17. In adults and particularly in those ages 45-74, the ratio reverses. In these age groups, the asthma is more prevalent in women (Toelle *et al.*, 2004).

2.4.3.3. Race:

Many investigations regarding literatures emerged the race of asthma as risk factor as trend of asthma status is more commonly in

African-American persons compared with Caucasian persons (Osuri,2002).

2.4.3.4. Family history of asthma:

Atopy is a genetic factor characterized by the body's production of immunoglobulin E (IgE) after exposure to common environmental allergens. If a person has a parent with asthma, he or she is three to six times more likely to develop asthma than someone who does not have a parent with asthma (Weiss,2001).

2.4.3.5.Premature birth or low birth weight(LBW) :

LBW is associated with childhood asthma and wheeze as proven by Arshad *et al.*, (2005) in a cohort study up to two years of age.

2.4.3.6.Viral respiratory infections in early childhood:

Infections of respiratory tract like *Chlamydia pneumoniae* are one of the factors responsible for onset of asthma, also they act as a trigger factor for asthma attacks (Gencay *et al.*,2001).

2.4.3.7.Lack of breast feeding:

A large number of researches illustrate that breast feeding protects against development of asthma , but this is not detected in all studies (Cengizler & Misirlioglu,2006).

2.4.3.8.Gastro-esophageal reflux disease (GERD) :

About 70% of patients with asthma have GERD. One of the earliest hypothesis of asthma pathogenesis suggested that the disease was due to occult (nocturnal) aspiration of gastric contents. Many studies have shown that GERD has a high incidence in asthmatics and that antireflux therapy can lead to a clinical improvement in severity of

asthma. More recent theories suggest that reflux of gastric acid causes distal esophageal irritation and this in turn causes bronchoconstriction mediated through the vagus nerve (Plottoová *et al.*,2001).In addition, theophylline that is used in asthma treatment can lead to decreased lower esophageal sphincter and thus reflux (Killjander *et al.*,1999).

2.4.3.9. Living in urban areas:

Asthma is more common in urban than rural areas. This may be related to breast feeding, non-immunization, and consumption of fermented milk products (Philip *et al.*,2008).Living in urban areas ,as compared with rural areas, is associated with increased prevalence of sensitization to aeroallergens. In addition, the prevalence of skin test reactivity is statistically higher in urban than rural area (Leynaert *et al.*,2001; Toelle *et al.*,2004).

2.3.4.10. ABO blood group system:

The ABO blood group system is widely credited to have been discovered by Austrian scientist, Landsteiner, who found three different blood types in 1900. Landsteiner described A, B, and O; Decastrello and Sturli discovered the fourth type, AB, in 1902. These groups are determined according to presence or absence of A or B antigens on the surface of red blood cells (Koers *et al.*,1989).

Recent studies attribute the cause of asthma to be related to ABO blood groups. Association has been found between asthma and blood group O.In addition, mild to moderate degree of severity of asthma was more characteristic in patients with group O. The risk for the

development of severe atopic bronchial disease was higher in patients with B phenotypes (Khetsuriani & Gamkrelidze,1995).

2.3.4.Pathophysiology of asthma:

2.3.4.1.Bronchial inflammation and constriction:

Asthma is an inflammatory disease. Inflammation is the approximate cause of airway hyperreactivity and variable airway obstruction in asthma, and is a universal finding in all asthmatic individuals (Maddox & Schwartz, 2002).It can be classified into extrinsic and intrinsic asthma according to the cause. Increased airway inflammation follows exposure to inducers such as allergens, viruses, and exercise. Increased inflammation lead to narrowing of airways and excessive mucus production which lead to coughing and other breathing difficulties, abnormal histopathologic lesions include edema, epithelial cells desquamation, and inflammatory cells infiltration (Zhu *et al.*,2001) as in figure (2-2). Reconstructive lesions including goblet cell hyperplasia , subepithelial fibrosis, smooth muscle cell and myofibroblast hyperplasia may lead to airway wall remodeling. Many studies have emphasized the multifactorial nature of asthma with interaction between neural mechanisms ,inflammatory cells (mast cells, basophils, macrophages, eosinophils, neutrophils, and lymphocytes), mediators (interleukins, leukotrienes, prostaglandins, and platelet activating factor) (Lilly,2005).

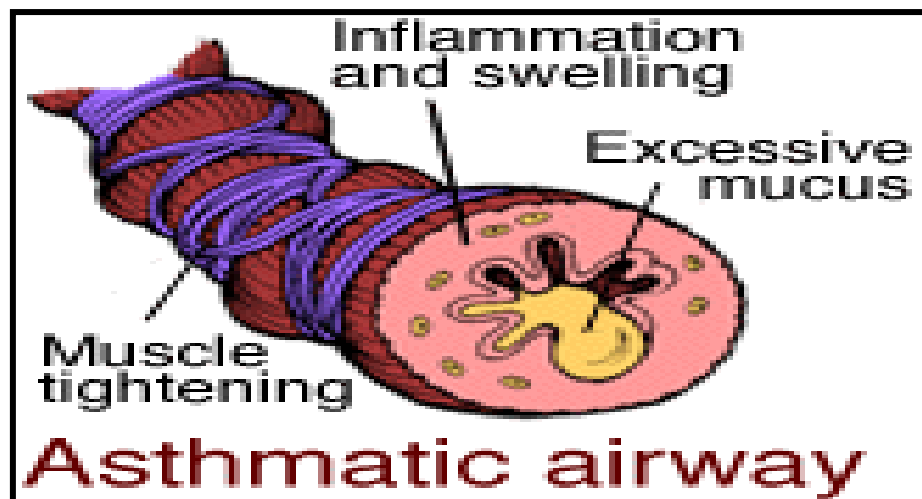


Figure (2-2): Common causes of asthma

(Porsbjerg *et al.*,2006).

2.3.4.2.Stimuli : There are many types of allergic stimuli and the most common stimuli types are:

2.3.4.2.1.Allergens:These allergens include various pollen, mould spores, cockroach, animal dandruff, house dust and various fungi. The most important allergen in house dust is provided by mites, notably *Dermatophagoides pteronyssinus*.

2.3.4.2.1. A. House dust mite (HDM) :

The house dust mite (HDM) is a cosmopolitan guest in human habitation. Mites remain in the mattresses, carpets, furniture, and bedding (Price *et al.*,1984; Zimmermann, 1989). It is one of the most common causes of allergy, implicated in allergic asthma, allergic rhinitis, allergic conjunctivitis, and allergic dermatitis. One of the most

important proteins responsible for allergic reaction is DerP, a protease digestive enzyme found in mite feces. Dust mite bodies, made of chitin, are also allergens (Platts-Mills & Carter,1997).

Dust mites are considered to be the most common cause of asthma and allergic symptoms worldwide. HDM are parasites belong to the phylum Arthropoda and the genus *Dermatophagoides*. The American HDM (*Dermatophagoides pteronyssinus*) and the European HDM (*D. farinae*) are two different species (Mitchell *etal.*,1985).



Figure(2-3): The house dust mite (*Dermatophagoides pteronyssinus*) (Arshad *et al.*,2001).

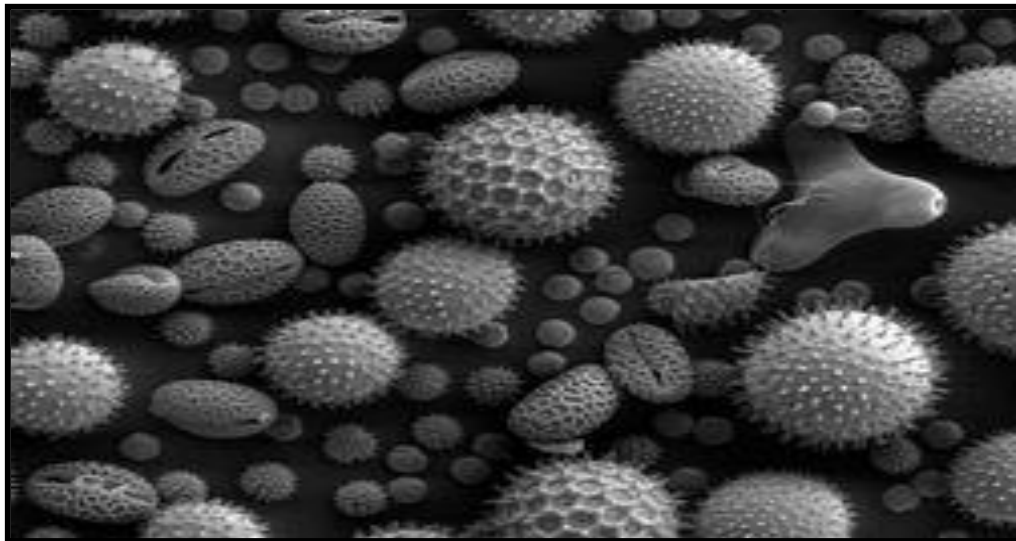
Dust mites can be transported airborne by minor air currents generated by normal household activities (Suggars,1998).

Some species of mites prefer to eat skin cells, a large component of household dust; others prefer flour dust (Korsgaard,1990). Dust mites have a rudimentary alimentary system (no stomach) and require most digestion to occur outside their body. For this reason they secrete enzymes and deposit the fungus *Aspergillus repens* on dust particles, to enable the fungus to pre-digest the organic matter with its enzymes. These enzymes are the most important part of mite dust in causing asthma and other allergic diseases (Glass *et al.*,1999). The allergens produced by various species of HDM are now well characterized. Three main classes, I , II, and III, are recognized. Class I , and III are thiol and serine proteases respectively, and weigh 25-29 Kd .Class II allergens are thought to derive from lysozyme (14Kd) (Stewart *et al.*,1999).

2.3.4.2.1.B.Pollen:

It is a fine to course powder produced by certain plants in order to reproduce. It is microscopic round or oval in shape (figure 2-4). In some species, the plant uses the pollen from its own flowers to fertilize itself. Other types may be cross pollinated; that is, in order for fertilization to take place, pollen must be transferred from the flower of one plant to that of another plant of the same species (Walter & Olmstead,2004). Each pollen grain contains vegetative cells (only one in most flowering plants but several in other seed plants) and a generative cell containing a tube nucleus (that produces the pollen tube) and a generative nucleus (that divides to form the two sperm

cells). The group of cells is surrounded by a cellulose cell wall and a thick, tough outer wall made of sporopollenin (Arshad *et al.*,2001).



Figure(2-4): Pollen from different plants (de Builderling *et al.*,2008) .

Pollen is transferred by insects or wind. The types of pollen that most commonly cause allergic reactions are produced by the plain-looking plants (trees, grasses, and weeds) (Ter-avanesian,2000).

2.3.4.2.2.Air pollution from volatile organic compounds includes perfumes, and perfumed products. Examples include : soap, shampoo, deodorants, hair gel, and cosmetics (Kuo *et al.*,2002; Johnston *et al.*,2002).

2.3.4.2.3.Medications include aspirin, beta-blockers, and penicillins (Jenkins *et al.*,2004).

2.3.4.2.4.Food allergies such as milk, peanuts, and eggs (Toelle *et al.*, 2004).

2.3.4.2.5.Exercise :

Asthma can also be made worse by physical exertion and this may occur to some extent in every asthmatic patient (Hauser *et al.*,2005).

2.3.4.2.6.Emotional stress :

Emotional factors play a major role in eliciting asthma attack in asthmatic patients, certain psychological factors can lead to severe and fatal asthma attacks like depression, separation in the family, and despair (Arshad *et al.*,2005).

2.3.4.2.7.Cold weather:

The cold weather makes it harder for asthmatics to breathe (Hauser *et al.*,2005).

2.3.4.2.8.Hormonal changes : Asthma may be worse in adolescent girls and adult women during menstrual cycles. During pregnancy , asthma may worse, or improves, or no change (Eseverri *et al.*,1998).

2.3.4.2.9.Respiratory tract infections (RTIs):

RTIs especially viral respiratory infections like sinusitis and influenza (flu) are among the most common stimuli that evoke acute attacks of asthma (Hogg, 2001;Osor,2002).

2.3.4.2.10.Various industrial compounds are those like dichloreamine and trichloreamine in the chlorinated swimming pools (Nemery *et al.*,2002).

2.3.4.3. Aetiology of Asthma :

Asthma is a multifactorial aetiological origin, arising from interaction of both genetic and environmental factors (Cengizlier & Misirlioglu, 2006).

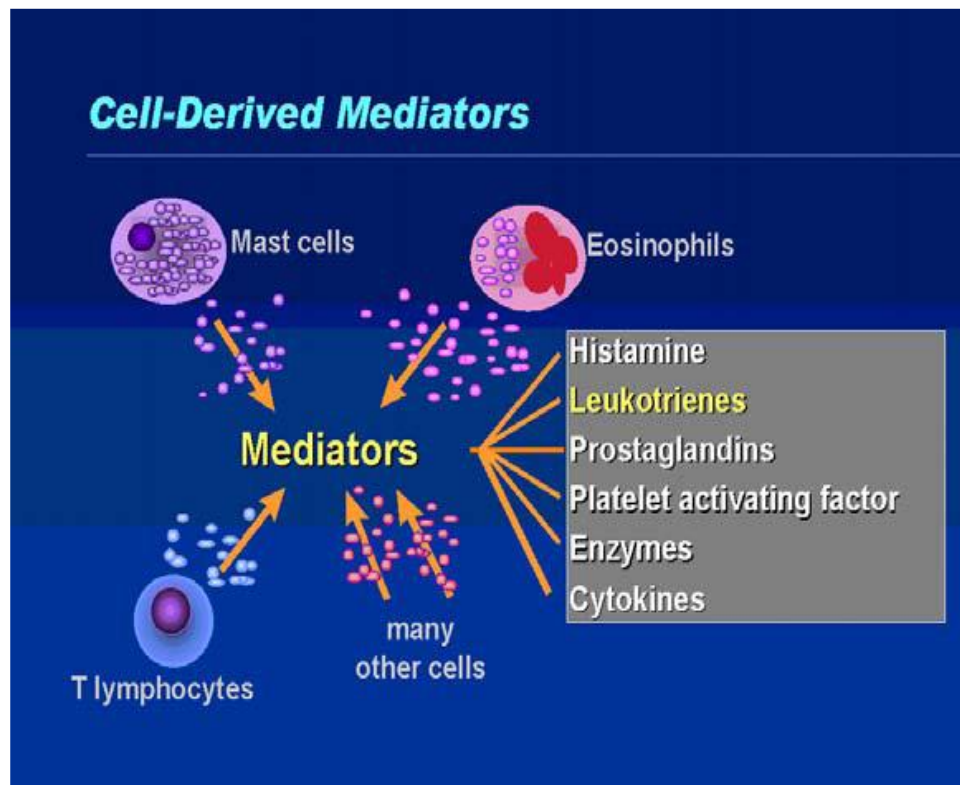
2.3.4.3. 1.Genetic Factors :

Asthma usually runs in families. Asthma which begins in childhood generally occurs in atopic individuals who have inherited tendency to produce significant amounts of IgE following exposure to allergens. If the mother only has asthma, the risk in children is 3.2 times more than the general population, if only the father has asthma, the risk is 2.9 times and 7 times more likely to develop asthma if both parents have asthma (Porth,2007).

2.3.4.3.2.Environmental factors :**2.3.4.3.2.1.Immunopathogenesis:**

Many environmental factors such as measles infection, BCG vaccine administration, and hepatitis A seropositivity can lead to predominance of T helper lymphocytes (Th2) in allergic asthma. This cell after stimulation by antigen-presenting cells (APCs) produces interleukin-4(IL-4) and IL-5.The IL-4 stimulates B cells to produce IgE, whereas, IL-5 stimulates maturation and chemotaxis of eosinophils. B cells are responsible for early phase response while eosinophils cause the late phase response. Eosinophils produce mediators and oxidants that lead to chronic injury and damaging effects in the airways. The allergic response in the airway is the result of a complex interaction of mast cells, eosinophils, T lymphocytes, macrophages, dendritic cells, and neutrophils (Figure 2-5). Inhalation-challenge studies with allergens reveal an early allergic

response (EAR), which occurs within minutes and peaks at 20 minutes following inhalation of the allergen (Porsbjerg *et al.*,2006).



Figure(2-5):Cells mediators showing mast cells, eosinophils, T lymphocytes and other cells and their mediators that trigger the allergic response (Cengizler & Misirlioglu,2006).

Clinically, the manifestations of the EAR in the airway include bronchial constriction, airway edema, and mucus plugging (Figure 2-2). These effects are the result of mast cell–derived mediators, four to 10 hours later, one sees the late allergic response, which is characterized by infiltration of inflammatory cells into the airway and

is most likely caused by cytokine-mediated recruitment and activation of lymphocytes and eosinophils (Dale and Federman,2003).

Antigen-presenting cells (ie, macrophages, dendritic cells) in the airway capture, process, and present antigen to helper T cells, which, in turn, become activated and secrete cytokines. Helper T cells can be induced to develop into Th1 or Th2 cells . Allergens drive the cytokine pattern towards Th2, which promotes B-cell IgE production and eosinophil recruitment. Subsequently, IgE binds to the high-affinity receptor for IgE, Fc-epsilon-RI, on the surface of mast cells and, with subsequent exposure to the allergen, the IgE is cross-linked. This leads to degranulation of the mast cells. Preformed mast cells mediators, such as histamine and proteases, are released, leading to the EAR (Majeed *et al.*,2008).

2.3.4.3.2.2.Hygiene Hypothesis:

The epidemiologic observation that asthma prevalence is greater in developed countries than developing countries was explained by hygiene hypothesis. This hypothesis proposes that airway infection and higher levels of exposure to animal allergens (e.g., farm animals, cat, dog) affect the balance between Th1 and Th2 in the airways. Early exposure to triggers that occurs more in rural areas shifts the balance toward Th1 and this protects against development of allergic disorders.

In urban Western society, lack of exposure shifts the balance toward Th2 and so more tendency toward allergic disorders (von Mutius, 2001).

2.3.4.5. Blood eosinophils and asthma association:

The eosinophil is inflammatory cell involved in pulmonary disorders, mainly allergic (Ulrick,1998).They have phagocytic function and they play a major role in defense against parasitic infections and in allergic reactions. The normal concentration in the blood is 1-4% of total leucocytes, while it is as high as 100-300 times in the tissues. Epidemiological findings have shown that blood eosinophil count appears inversely related to the level of pulmonary function.

Population based data have shown that peripheral blood eosinophil is related to asthma prevalence and acute attacks, bronchial hyperresponsiveness and decreased lung function (Griffin *et al.*,1991).

2.3.5.Clinical features of asthma :

2.3.5.1.Symptoms :

Some or all of the following may be present in patients with asthma: dyspnea (shortness of breath), wheeze, 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 tract infection or after exposure to allergens like pollen, house dust mite, animal dander and so on (Rodrigo *et al.*,2004).

2.3.5.2.Signs :

Physical examination of patients with asthma reveals rapid breathing (tachypnea), rapid heart rate (tachycardia), prolonged

expiratory phase, rhonchi (sounds audible through a stethoscope), and overinflation of the chest (Hauser *et al.*,2005). Asthma can be classified according to clinical features into mild intermittent, mild persistent, moderate persistent, and severe persistent (table 2-1).

Table (2-1) : Classification of asthma severity by clinical features before treatment (Dale & Federman, 2003.

Type	Episodes of Symptoms	Nocturnal symptoms	FEV1 or PEF*
Mild Intermittent	≤ twice a week	≤ twice a month	> 80% predicted, brief exacerbations.
Mild persistent	> twice a week	> twice a month	> 80% predicted, once a day,
Moderate persistent	Daily	≥ Once a week	60% - 80% predicted, attacks affect activity
Severe persistent	Continuous throughout the day	Frequent	< 60% predicted, limited physical activity

*FEV1:Forced expiratory volume in first second, PEF:Peak expiratory flow.

2.3.5.3. Blood hemoglobin concentration and asthma relationship:

Hemoglobin (Hb) is a protein especially adapted for gas transport to and from the lungs. It is composed of four globin chains, each containing an iron-containing porphyrin pigment termed haem (Boon *et al.*,2006).Patients with mild to moderate asthma usually have normal level of blood Hb. This might be due to the attacks of asthma are

usually intermittent, therefore there is no sustained rise of blood hematocrit (Gordon,2008).

2.3.5.4. Blood pH and asthma association:

Patients with mild to moderate asthma usually have respiratory alkalosis ($\text{pH} > 7.45$). This occurs due to alveolar hyperventilation (Fall, 2000). Alveolar hyperventilation leads to a decreased PaCO_2 (partial pressure of CO_2) level (hypocapnea). In turn, the decrease in PaCO_2 level increases the ratio of bicarbonate concentration (HCO_3^-) to PaCO_2 and increases the pH level (Mountain *et al.*, 1990). Hypocapnea occurs when the lungs remove more carbon dioxide than is produced in the tissues (Gluck, 1998).

2.3.6. Indices of Asthma Diagnosis :

Asthma is simply defined as reversible airway obstruction. The basic measurement is peak flow rates and the following diagnostic criteria are used by the British Thoracic Society (Pinnock & Shah, 2007) :

- $\geq 20\%$ difference on at least three days in a week for at least two weeks.
- $\geq 20\%$ improvement of peak flow following treatment like:
 - 10 minutes of inhaled β -agonist (like salbutamol).
 - Six weeks of inhaled corticosteroid (like beclomethasone).
 - 14 days of 30 mg prednisolone.
- $\geq 20\%$ decrease in peak flow following exposure to a trigger (e.g., exercise).

In many cases, asthma can be diagnosed depending on a typical finding in a patient's clinical history and examination. Asthma is strongly suspected if a patient has eczema or other allergic disorders which suggest general atopic condition or has a family history of asthma (Douglass & O'Hehir,2006).

For initial diagnostic purposes, spirometry rather than peak flow meter should be performed. Spirometry typically shows obstructive airway disease with reduced expiratory flow that improves with bronchodilator therapy. It shows reduced expiratory volume at first second (FEV1) , reduced forced vital capacity(FVC), and reduced FEV1/FVC ratio (Oga,2001).

Chest X-ray is not useful in diagnosis, but it is performed to exclude other conditions and if complications like pneumonia or pneumothorax were suspected. It commonly shows hyperinflation which has no diagnostic or therapeutic value. If the diagnosis is in doubt, challenge test may help to identify airway hyperreactivity as the cause of the symptoms. Agents commonly used for challenge test are methacholine or histamine (Pinnock & Shah, 2007).

2.3.7.Managent of asthma :

2.3.7.1.Patient Education:

This involves the patient understanding the nature of asthma, proper use of drugs, and use of peak flow meter which provides the patients with an objective measure of airflow obstruction (Aw *et al.*,2001) .

2.3.7.2.Avoidance of precipitating factors :

The most effective treatment for asthma is identifying triggers like smoke, allergens, and aspirin and trying to avoid exposure to these agents but this is not always possible (Boon *et al.*,2006).

2.3.7.3.Medical treatments :

The specific medical treatments depend on the severity of illness and the frequency of attacks. Specific treatments are broadly classified into relievers, preventers (Seema & Wasserman,2005)..

2.3.7.3.1.Relief Medications (Relievers) :

These drugs produce symptomatic control of wheeze and shortness of breath. For patients with occasional attacks, only bronchodilators are used. For those with mild persistent asthma, low-dose inhaled glucocorticosteroids (GCs), or theophylline can be used. For those with daily attacks, a higher dose of GC with long-acting inhaled β_2 -agonist can be used. In severe cases, oral GCs are added (Pinnock & Shah, 2007).

Relievers include (Joshi,2000) :

- A. Short-acting selective β_2 -agonists such as salbutamol or terbutaline.
- B. Non-selective adrenergic agonists such as inhaled epinephrine or epinephrine tablets.
- C. Anti-cholinergic medications such as ipratropium bromide.

2.3.7.3.2.Preventive medications (Preventers) :

These drugs suppress inflammation and reduce swelling of the airways lining.Preventive drugs include : Inhaled GCs , leukotriene modifiers like montelukast, mast cell stabilizers like cromoglicate,

anticholinergics, methylxanthines like aminophylline, antihistamines, omlizumab(IgE blocker), methotrexate, anti-acids for gastro-esophageal reflux disease (GERD) (Seema & Wasserman,2005).

2.3.7.4.Allergen Immunotherapy(AI) :

2.3.7.4.1.Historical overview :

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 (Huggins & Looney, 2004).

Allergen immunotherapy was developed for a long period before immune mechanisms involving IgE antibodies were defined. Immunotherapy was first used successfully in 1911 by Leonard Noon, who noticed that in many people the onset of allergic symptoms coincided with the pollination of grass in England. He found that intradermal injections of an extract of grass pollen relieved the signs and symptoms that patients experienced during the grass pollen season (Goddie & Palmar,1986). Since that time, the use of immunotherapy has greatly increased. According to the American Academy of Allergy and Immunology, approximately 33 million injections are given per year in the United States (Joshi *et al.*,2003).

2.3.7.4.2.Clinical indications:

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 (Mathew & James,2007).

2.3.7.4.3.Contraindications :**A. Absolute contraindications:**

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 (Lockey,2001).

B. Relative contraindications :

These include pregnancy (maintenance injections can be continued during pregnancy), small children (less than five years age old), and elderly patients (Weiner,2006).

2.3.7.4.4.Efficacy :

The Allergen immunotherapy is effective in allergic asthma (Nelson, 2000). Abramson *et al.*, (1999) evaluated 20 studies performed in the period between 1990 and 1995 , 12 trials of immunotherapy for HDM allergy and 8 trials for pollen allergy. They observed significant improvement in asthma symptoms scores, people receiving immunotherapy were less likely to report a worsening of asthma symptoms than those receiving placebo and were less likely to

require medications. Basomba *et al.*, (2002) conducted a double-blind, placebo-controlled study in 55 asthmatic patients sensitized to HDM. The study continued 12 months. In nearly half of the patients actively treated, there was a reduction in their symptoms and medication scores by at least 60% versus only 12% of patients receiving placebo treatment. Percentage of healthy days in the immunotherapy group rose from 10.5% before treatment to 64.5% after treatment. Reduction in sensitivity was demonstrated by skin prick test response, late-phase response after intradermal testing and bronchial challenge test result in the immunotherapy group. Serum levels of IgG increased throughout the treatment whereas serum IgE levels showed only transient initial increase. No change in these parameters was observed in the placebo group.

2.3.7.4.5.Immunotherapy for Secondary Prevention :

Allergen immunotherapy (AI) has the ability to modify the natural course of allergic diseases. Pajno *et al.*, (2001) studied 134 children (age ranged from 5-8 years) who had intermittent asthma with or without rhinitis, with single sensitisation to mite allergen. AI was received by 80 children and the remaining 54 children were treated with medications only and were considered the control group. AI with mite was administered for 3 years and all patients were followed for 6 years ;123 children completed the follow-up study. About 52 out of 69 children (75.4%) in the IT group showed no new sensitization ,compared with 18 out of 54 children (33.3%) in the control group. Eng *et al.*,(2002) examined a group of patients who had received specific

injection immunotherapy (SIT) to grass pollen 6 years after discontinuation of treatment. Scores for symptoms remained lower in the group treated with SIT, 61% had developed new sensitization to perennial allergens compared with 100% in the control group, so, SIT in children with pollen allergy reduces onset of new sensitization .

2.3.7.4.6. Long-term benefits :

Allergen Immunotherapy has been shown , in different studies , to confirm long-term benefits following discontinuation. In one double blind placebo-controlled study, 3-4 years grass pollen immunotherapy was shown to result in sustained reduction in symptoms and medication use for at least 3 years following discontinuation (Abramson *et al.*, 1995). It also reduces the onset of new sensitivities in children. In a prospective randomized controlled trial of pollen immunotherapy in children with seasonal allergic rhinitis , development of asthma was followed up for 5 years. There was a marked reduction in the risk of development of asthma compared to controls (Pajno *et al.*, 2001) .

2.3.7.4.7. Factors affecting allergen vaccine potency :

2.3.7.4.7.1. Storage of allergen vaccine :

Vaccines should be stored at 2-8C° because storage at room temperature result in loss of vaccine potency within weeks (Huggins & Looney ,2004).

2.3.7.4.7.2. Time : Vaccines should be checked for expiration date (Olaguibel *et al.*, 1998).

2.3.7.4.7.3. Volume of the vial :

A volume effect can occur as a result of adherence of the allergen to the surface of the vial ; the larger the surface area of the vial , the more allergen will be lost. Glycerol and human serum albumin (HSA) are used to reduce volume effect (Huggins & Looney, 2004).

2.3.7.4.7.4. Concentration :

Highly concentrated vaccines are more stable than dilute vaccines (Bousquet and Michel, 1994).

2.3.7.4.7.5. Diluent used:

Concentrated extracts must be diluted with an appropriate sterile diluent prior to first use on a patient for treatment or intradermal testing. Diluents commonly used are saline with human serum albumin (HSA), buffered saline or saline (Pichler *et al.*, 2001).

2.3.7.4.7.6. Type and number of allergens :

Patients may have multiple sensitivities to different allergens , so vaccines containing mixture of these allergens can be prescribed. This results in reduction the concentration of each allergen and certain allergens may interact (Reha & Ebru, 2007).

2.3.7.4.8. Methods of administration and procedure :

Allergen immunotherapy should only be performed by trained staff in an allergy clinic. Facilities for vaccine storage and resuscitation should be available . Allergen immunotherapy should only be given in the immediate presence of a physician. Allergen immunotherapy can be given by two ways :

Injection route (Allergen injection immunotherapy), and sublingual route (Ana *et al.*,2008).

2.3.7.4.8.1. Allergen injection immunotherapy(AII):

The entire test procedure takes less than one hour from the time patients arrive to the clinic until they leave. Before injections has been done, complete history was taken from the patient with full physical examination. Records should include the date, volume, and the dose of vaccine given. Peak expiratory flow should also be recorded as well as any delayed local or systemic reactions following previous injection should be recorded. Adrenaline should be immediately available. The patients remain for at least 30 minutes following injections. AII is divided into two phases : phase one is diagnostic testing, called allergy skin test and phase two is subcutaneous immunotherapy (Cevdet *et al.*,2007).

2.3.7.4.8.1.A.Allergy skin test :

It is an allergy test used to diagnose the type of allergic substance. Some drugs interfere with test results and should not be taken by the patients. These drugs include (Sarpong and Karrison,1998) :

a.Antihistamines : Antihistaminic drugs can prevent allergic reactions. They block the action of histamine which is released during allergic reactions.

b.Tricyclic anti-depressants : These drugs may lead to more skin sensitivity to light than normal and may affect the accuracy of skin test results.

c.Corticosteroid cream : They can suppress reactions in the areas they are applied.

Allergy skin tests include the following types (Rosa *et al.*,1995):

A.Prick or scratch test :

Tiny amounts of different allergens are placed on the skin , and a series of tiny scratches or needle pricks forces the allergens into the skin. It is less likely to produce anaphylaxis. Both tests (prick & scratch) are very good to identify specific allergies, but they are less sensitive than intradermal test and sometimes show no reaction to an allergen even when an allergy is present.

B. Intradermal test :Tiny amount of an allergen is injected through the intradermal skin layer. It is more sensitive but less specific than prick or scratch test.

C.Patch test : Patch containing an allergen is placed on the skin of patient for 24 to 72 hours. It is used to identify allergic contact dermatitis.

2.3.7.4.8.1.B.Subcutaneous immunotherapy:

Conventional schedule: Conventional schedule include two phases (Francis *et al.*,2008) :

A. Build-up phase: Injections are given weekly starting with very low dose of an allergen and gradually increased to reach the maintenance phase after three months.

B. Maintenance phase: The maintenance dose is repeated monthly for 3-5 years. The maintenance dose represents the highly effective dose that can be tolerated.

2.3.7.4.9. Adverse reactions :

Itching, swelling and redness at the site of injection are expected. Systemic reactions such as hives or anaphylaxis occur rarely and need to be treated immediately. If systemic reactions occur, the dose should be adjusted to a safe level. Lockey, (2001) have found, after statistical review of literature about systemic reactions following AI ,that severe systemic reactions occurred in less than 1% of patients receiving conventional immunotherapy in the united states. From 1985 to 1993 in the united states, 52.3 million administrations of immunotherapy resulted in 35 deaths. This is equal to a mortality incidence of less than one per million (Lue *et al.*,2001).

Adverse reactions and suggested treatment can be shown in the table (2-2).

Table (2-2).Adverse reactions and suggested treatment (Huggins & Looney,2004)

Adverse reactions	Suggested treatment
Local reactions :common, occur at the injection site, IgE mediated, manifested by wheal, flare, and pruritus.	Local cold pack, oral antihistamine, and topical steroid.
Large local induration : occurs at injection site, IgG complex reaction, manifested by pain, tenderness, and hard swelling.	Oral anti-histamines and oral steroid.
Systemic reactions (anaphylaxis): urticaria, angioedema, hypotension, vomiting, dyspnea	Tourniquet above injection site ,aqueous epinephrine injection, diphenhydramine,i.v. fluid.

2.3.7.4.10.Precautions:

The vaccine should not be given intravenously and should not be administered by the patient himself. Allergen immunotherapy should not be given to patients receiving beta blockers because they are not responsive to β -adrenergic drugs used to treat anaphylaxis that may happen. There is no evidence of carcinogenesis, mutagenesis, or impairment of fertility in human from mite extracts. Allergen immunotherapy is contraindicated in patients with autoimmune disorders because AI causes conversion of Th1 toward Th2 which is already increased in patients with autoimmune disorders (Huggins & Looney, 2004). Similarly, patients with unstable asthma who have a forced expiratory volume in one second (FEV₁) or a peak flow value below 70 percent of predicted value should not receive immunotherapy because of their decreased respiratory reserve secondary to the underlying disease.

2.3.7.4.11.Duration of Immunotherapy:

Studies have demonstrated both short-and long-term benefits of immunotherapy. These benefits persist even after discontinuation of immunotherapy. One study demonstrated that IgE level ,the signs and symptoms,and the response to histamine challenge showed continued improvement three to five years after discontinuation of immunotherapy (Hedlin *et al.*,1995).In general, immunotherapy is administered for three to five years(Canadian Society of Allergy and Clinical Immunology,1995).

In most studies, improvement in symptoms occurs within six months to a year of allergy shots, if no improvements after this time, the shots are stopped. If the treatment is effective, the shots often continue three to five years, until the patient is symptom-free or the symptoms can be controlled with mild medications for one year (Joshi *et al.*,2003). If the shots are stopped prior to a total of three years, the allergic symptoms typically return more quickly (Ferrer & García-Sellés,2003).

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