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STUDY OF SERUM INTERLEUKINS (IL 13, IL 9, TNF) IN ASTHMATIC PATIENTS WITH MITE ALLERGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN
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BY

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We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

Master Thesis

STUDY OF SERUM INTERLEUKINS (IL 13, IL 9, TNF) IN ASTHMATIC PATIENTS WITH MITE ALLERGY

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Asthma is a problem of a genetic inflammatory disease of the respiratory tract-airways in which clinical disease progression is influenced by environmental factors. Chronic airway inflammation, usually with eosinophil infiltration, is one of the hallmarks of asthma. Most asthmatics can be successfully treated with conventional medicine according to their severity, but in certain extreme cases, even with prolonged treatment, asthma remains uncontrolled, which is known as'refractory asthma. A new biologic-based therapy strategy for severe refractory asthma has been created based on knowledge of the molecular pathways of airway inflammation in asthma produced by enhanced Th2-type responses, eosinophil activation, and allergic reactions. The first biological preparation approved to treat asthma was humanized anti-human IgE antibody (anti-IgE; omalizumab). Treatment with anti-IgE (anti-IgE therapy) has been recognised as a new therapeutic option for severe allergic asthma in adults since 2009 and children since 2012, based on clinical evidence, and has been demonstrated to have a 60% effectiveness rate. The TH2 cytokine family's interleukin (IL)-9 and IL-13 have recently been suggested as a key component in determining mucosal immunity and susceptibility to atopic asthma. TNF- is a key player in the establishment of the Th2 cell response to allergens ingested.

2021, 65 pages

Keywords: Asthma, Dust Mites, Interleukin-13, Interleukin-9, Tumor Necrosis Factor Alpha.

ÖZET

Yüksek Lisans Tezi

AKAR ALERJİSİ OLAN ASTIMLI HASTALARDA SERUM İNTERLEUKİNLERİNİN (IL 13, IL 9, TNF) ÇALIŞMASI

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Astım, klinik hastalık progresyonunun çevresel faktörlerden etkilendiği, solunum yolu-hava yollarının genetik bir inflamatuar hastalığı sorunudur. Genellikle eozinofil infiltrasyonu ile birlikte kronik hava yolu inflamasyonu, astımın ayırt edici özelliklerinden biridir. Çoğu astımlı, şiddetine göre geleneksel tıpla başarılı bir şekilde tedavi edilebilir, ancak bazı aşırı durumlarda, uzun süreli tedavi ile bile astım kontrolsüz kalır ve buna 'dirençli astım' denir. Artmış Th2-tipi yanıtlar, eozinofil aktivasyonu ve alerjik reaksiyonlar tarafından üretilen astımdaki hava yolu inflamasyonunun moleküler yolakları bilgisine dayalı olarak, şiddetli dirençli astım için biyolojik temelli yeni bir terapi stratejisi oluşturulmuştur. Astımı tedavi etmek için onaylanan ilk biyolojik preparasyon, insanlaştırılmış anti-insan IgE antikoruydu (anti-IgE; omalizumab). Anti-IgE ile tedavi (anti-IgE tedavisi), klinik kanıtlara dayalı olarak 2009'dan beri yetişkinlerde ve 2012'den beri çocuklarda şiddetli alerjik astım için yeni bir tedavi seçeneği olarak kabul edilmiş ve %60 etkililik oranına sahip olduğu gösterilmiştir. TH2 sitokin ailesinin interlökin (IL)-9 ve IL-13'ünün yakın zamanda mukozal bağışıklığın ve atopik astıma duyarlılığın belirlenmesinde anahtar bir bileşen olduğu öne sürülmüştür.

2021, 63 sayfa

ANAHTAR KELİMELER: Astım, Toz akarları, İnterlökin-13, İnterlökin-9, Tümör nekroz faktörü alfa

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Hiyam Sameer ABDULAMEER ALSAADI

Cankiri - 2021

Contents

LIST OF ABBREVIATIONS

Abbreviation	Expantion
APC	Antigen-Presenting Cell
B cell	Bone marrow- or Bursa-derived cells
CCGF	C-C Motif Chemokine Growth Factor
CCL	C-C Motif Chemokine Ligand
CCR	C-C Motif Chemokine Receptor
CD4+	Cluster of Differentiation 4
DC	Dendritic Cell
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HDM	House Dust Mites
IgE	Immunoglobulin E
IL	Interleukin
LPS	Lipopolysaccharide
MMPs	Matrix Metalloproteinases
NK	Natural Killer
PAF	Platelet Activating Factor
QTL	Qualitative Trait Locus
T cell	Thymus cells
Th	T-helper
TNF	Tumor Necrosis Factor
TSLP	Thymic Stromal Lymphopoietin

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1. INTRODUCTION

Allergy is an immune system over- or hypersensitivity reaction to specific environmental substances known as allergens, which is normally harmless to most people. Pet dander, dust mites, food, pollen, insects, and chemicals are all common allergens. (Göney *et al.* 2017, Austin, 2015). Asthma, allergic rhinitis, and conjunctivitis are some of the most frequent allergic reactions to house dust mites. (Doğan, Aycan, Miman, Atambay, & Daldal, 2008). Depending on the results of 85 of atopic-dermatitis-patients and 92 of asthmatic patietns,Terreehorst and colleagues discovered that those patients had a history with rhinitis infection in an experimental-study which was done in 2002 on more that three handred patients with atopic disorders. implying that these patients suffer from a high frequency of nose symptoms due to house dust mite sensitivity. Also, they found that these three types of atopic diorders have a significantly connection assocition, especilly between respiratory allergic diorders. (Terreehorst et al., 2002).

Dust vermin are considered the foremost visit asthma allergen, and whereas a few individuals have a fundamental tidy sensitivity, others endure from atopic dermatitis, in some cases known as skin inflammation, which causes unpleasant tingling and redness when they come into contact with bugs. Asthma has been linked to both Dermatophagoides farinae Hughes (the American house tidy vermin) and Dermatophagoides pteronysinus Trouessart (the European house tidy vermin) of the Pyroglyphidae (Acari) family of mites. The regular populaces or the exact nature of tidy vermin thickness in houses is basic for lessening their development and understanding their association in tidy sensitivity (Sarwar, 2020).

Allergic rhinitis is an allergic reaction that causes inflammation of the nasal mucosa. As commonly known, rhinitis is not serious in comparing to asthma, but on the other hand, is considered quite burdensome which mean affects the person's health. Allergic rhinitis also induced by HDM (house dust mites) allegens, with symptoms occurring on a regular or irregular basis. Atopy is common in people who suffer from allergic rhinitis or asthma, and it runs in their families. Atopy is a genetic predisposition to cause acute hypersensitivity reactions to typically harmless chemicals, usually mediated by immunoglobulin E (IgE) antibodies. In several scientific studies, atopy has been presented as main link between allergic illnesses such skin rashes (dermatitis), sever sneezing (rhinitis), asthma, and conjunctivitis. (Terreehorst et al., 2002). Allergic conjunctivitis is an inflammation of the eye's conjunctiva membrane induced by allergens in the air. Atopic dermatitis, on the other hand, is skin irritation caused by a hypersensitive reaction.

In Allergy ,rhinitis and asthma are most common in children, adolescents, and young adults. Symptoms may decline as people get older, but they might last a lifetime. If an individual is exposed to an allergen once and becomes sensitized to it, they are said to have developed an allergy as a result of the initial exposure. An allergic reaction occurs

when an allergen irritates the nasal mucosal membrane. APCs display allergens to T helper-2 (TH2) lymphocytes, which TH2 cells then release interleukin-4 (IL-4) and interleukin-5 (IL-5), inflamatory cells, and draw basophils and eosinophils to the airways. White blood cells called granulated eosinophils have a major role in asthma (Small, 2003; Hogan et al., 2008).

These granulocytes are observed in rare way in the airways of the healthy people, but approximately half of asthmatics, the levels of the previous cells in their blood and tissue are dramatically raised (Wenzel et al., 1999). Degranulation is frequently accompanied by increased eosinophilic inflammation of the airways, which is largely related with greater asthma severity. Eosinophils rapidly release immunomodulatory cytokines when stimulated. (Davoine & Lacy, 2014). One of the best-known eosinophil activators is platelet activating factor (PAF), a powerful lipid mediator that acts on the PAF receptor and other pathways to generate eosinophilia. In addition to IL-4, IL-5, and IL-13, eosinophils manufacture and store a number of other Th2 cytokines (Davoine & Lacy, 2014).

In eosinophilic inflammatory disorders, both IL-9 and IL-13 tilt the immune response to a Th2 phenotype. (Schmid-Grendelmeier et al., 2002; Zhou, McLane, & Levitt, 2001). Although the role of these Th2 cytokines in immunity and asthma is well established, their unique intracellular trafficking mechanisms in eosinophils have yet to be described. Cytokines aid in the regulation of asthmatic airway inflammation by encouraging inflammatory cell growth, differentiation, reinforcement, priming, activation, and survival. Individual cytokines may have overlapping cell regulatory actions, and complex networks of cytokines may function. Asthma is thought to be a T helper cell type 2 (Th2) illness, with interleukin 4 (IL-4), IL-5, and IL-13 cytokine profiles. Other cytokines assumed to be associated with a Th1-type profile have been linked to the inflammatory response in asthma, according to evidence. TNF-a is a Th1 cytokine that has been shown to play a function in asthmatic airway inflammation in both in vitro and in vivo investigations. TNF- expression has been found to be higher in asthmatic airways than in normal patients' airways. (Bradding et al., 1994).

In chronic allergic asthma, the allergen stimulates submucosal mast cells in the lower airways, causing bronchial constriction and an increase in fluid and mucus discharges, making breathing difficult. This is due to a late phase reaction in which active mast cells and eosinophils continuously create and release chemical mediators like cytokines and leukotrienes. Mast cells also produce IL-5, this will also lead to produce of other cells which are eosinophils which inturn produce other mediators which play a role in inflamation. Allergic rhinitis ; especially the chronic type; exhibits a similar late-phase reactivity (Abbas, Lichtman, & Pillai, 2014; Vogel, 1997). House dust mite allergy can be diagnosed in a variety of ways. Skin-prick testing, in which small amounts of different allergen extracts are plunged, generally in the upper arm, to measure the patient's reaction, is perhaps the most popular approach. Allergen extracts are usually made from normal

components, they still have a number of drawbacks, despite its nature quality. The most common types of their components are non-allergenic proteins, in addition to others like proteolytic-enzymes, which might cause the extract to degrade and lose its efficacy. Second, the makeup and amount of allergens can change. Third, allergies from other sources can contaminate them. (M. D. Chapman et al., 2000).

Aim of the study is:

- Estimation of total and specific IgE, IL-13, IL-9 and TNF-α in serum and eosinophil count in Iraqi asthmatic patients.

2. LITERATURE REVIEW

2.1. Allergy

Allergies are immune system overreactions to something that would normally be harmless. Varied allergies have variable triggers, symptoms (ranging from mild to life-threatening), and treatments, as well as different frequencies of occurrence in the population. Some allergies exhibit symptoms that are similar to those of other illnesses. (Austin et al., 2015).

2.1.1. Mechanism of Allergy

When a person is exposed in first time to an allergen, the allergen is transported to the T lymphocytes in the closest lymph node by dendritic cells. Any substance that isn't allergic to the person is ignored and eliminated. If the material is recognized by the lymphocytes as an allergen, a complex chain of immunological reactions ensues, culminating in an allergic reaction. (Sears and Sears, 2015).

First encounter to the allergen, the Th2 type of T cells in that lymph node proliferate. Th2 lymphocytes are the immune cells that are principally responsible for programming the rest of the immune system to react in an allergic manner, as you may have already forgotten. They accomplish this by secreting cytokines in order to activate B lymphocytes, which create IgE antibodies that will react to the allergen the next time it enters the body. This cytokine-mediated activation happens throughout the body, priming and loading B cells with IgE antibodies in preparation for release at any time. The cytokines, however, do not stop there. They also organize mast cells, basophils, and the rest of the innate immune cells found in bodily tissues and mucous linings to react to that particular allergen. (Sears and Sears, 2015).

Second encounter to the allergen, the IgE antibodies are the first to recognize the allergen and bind to it. The antibody/allergen combination is subsequently recognized by the innate immune cells, which respond in one of two ways: They release substances (histamine, acids, and enzymes) that induce swelling, stinging, and irritation in the surrounding tissues, as well as additional cytokines that draw allergy-responding immune cells to the area. T and B lymphocytes are triggered, and the allergic reaction spreads throughout the body to varied degrees, depending on the severity of the allergy. Histamines also elicit sneezing by activating sensory nerve cells in the area, which alert the brain to the allergen's existence. (Sears and Sears, 2015). (Figure 2.1).

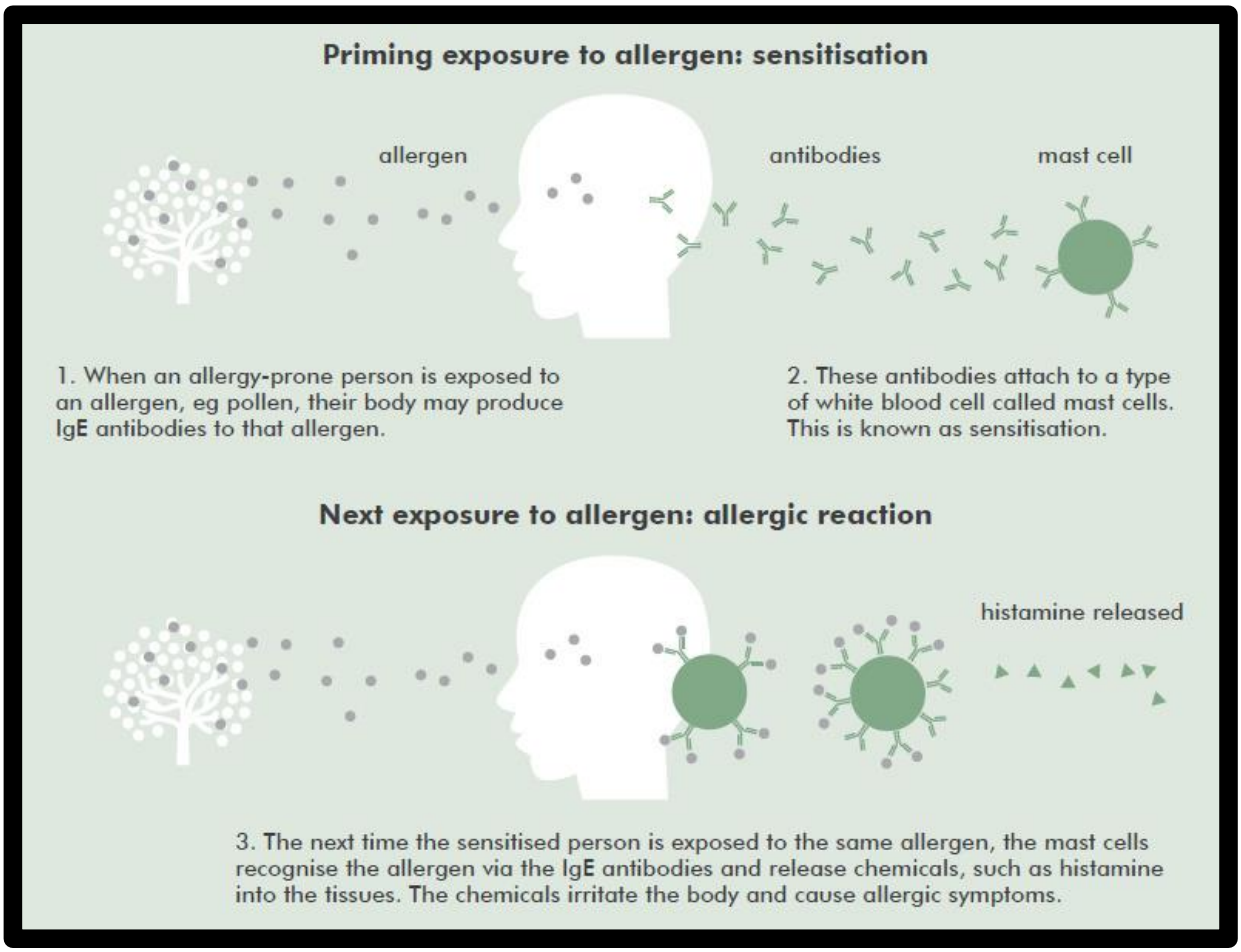

Figure 2.1: Mechanism of Allergy. (Austin et al., 2015).

2.2. Asthma

Asthma is a chronic, non-communicable condition that affects both children and adults and is characterized by a wide range of respiratory symptoms and airflow limitations: such as wheezing, shortness of breath, chest tightness, and cough that change in intensity over time (Pani et al., 2018). The presence of reversible airflow restriction is the required-sign for the diagnosis of asthma. Inflammatory cells such as eosinophils, neutrophils, lymphocytes, and mast cells, as well as airway components, are involved in airway inflammation, bronchial epithelium cells, fibroblasts, and bronchial smooth muscle cells, as well as a variety of humoral components Airway remodeling occurs as a result of persistent airway inflammation, resulting in irreversible airflow restrictions. (Lambrecht et al., 2019; Peters et al., 2020). (Figure 2.2).

2.2.1. Asthma Phenotypes

Asthma is a heterogeneous disease “which mean is a medical word for a medical illness that has numerous causes, with numerous physiological processes”. The different

physiological charcteristics in addition to clinical characteristics are describing the asthma forms “or as named asthma phenotypes” .

There are distinct groups of asthmatic traits that can be identified. These clusters are generally referred to as asthma phenotypes. For patients with severe asthma, phenotype-guided treatment is available. To yet, however, there has been no conclusive link established between discrete pathogenic features and specific clinical patterns or therapeutic responses phenotypic categorisation has not been proven to be clinically effective in asthma and additional research is needed. (Bel, 2004; Moore et al., 2010; Wenzel, 2012).

There are several types differ and similar clinically and phenotypically of asthma, the most common of them are including:

• *Allergic asthma*: This is the most well-known asthma phenotype, which usually begins in childhood and is linked to a history of allergic disease such as eczema, allergic rhinitis, or food or chemical allergies in the family. Before treatment, these individuals' produced sputum frequently reveals eosinophilic airway inflammation. Inhaled corticosteroid (ICS) treatment works well for patients with this asthma phenotype.

• *Non-allergic asthma (or Intrinsic)*: About 10% to 33% of people affected by this of asthma, and typically develops later than allergic asthma, with a female preponderance. In many situations, nonallergic asthma appears to be more severe than allergic asthma, and it may be less responsive to conventional treatment. Few of asthmaitic patients may not have association with allergy. These individuals' sputum may have a neutrophilic, eosinophilic, or inflammatory cell profile with just a few inflammatory cells (paucigranulocytic). Non-allergic asthma patients generally have a worse short-term response to ICS.

• *Adult-onset (late-onset) asthma*: some adults, especially ladies, display with asthma for the primary time in grown-up life. These patients tend to be non-allergic, and regularly require higher measurements of ICS or are moderately headstrong to corticosteroid treatment. Adult-onset asthma, too known as late-onset asthma (LOA), is on the rise as the populace ages. The nearness or nonappearance of eosinophilic aggravation fundamentally partitions the phenotype of LOA into two sorts, LOA connected to T-helper (Th)2 and non-Th2 cells. Word related asthma (i.e. asthma due to exposures at work) ought to be ruled out in patients displaying with adult-onset asthma.

• *Asthma with persistent-airflow-limitation*: Some people with long-term asthma acquire persistent or incompletely reversible airway restriction. This is assumed to be linked to remodeling of the airway wall.

• *Obesity associated Asthma*: Obesity has often been recognized as an issue key in asthma patients, having its own phenotypic and endotype. This link suggests there is a link

between metabolic and inflammatory dysregulation. The specific organ-organ, cellular, and molecular relationships, on the other hand, remain unresolved. A few overweight-patients with asthma have noticeable respiratory side effects and small eosinophilic respiratory irritation.

Despite the lack of data on the natural history of asthma after diagnosis, one research revealed that around 16% of people with newly diagnosed asthma may have remission within 5 years. (Peters, 2014; Hirano and Matsunaga, 2018; Westerhof et al., 2018; Miethe et al., 2020).

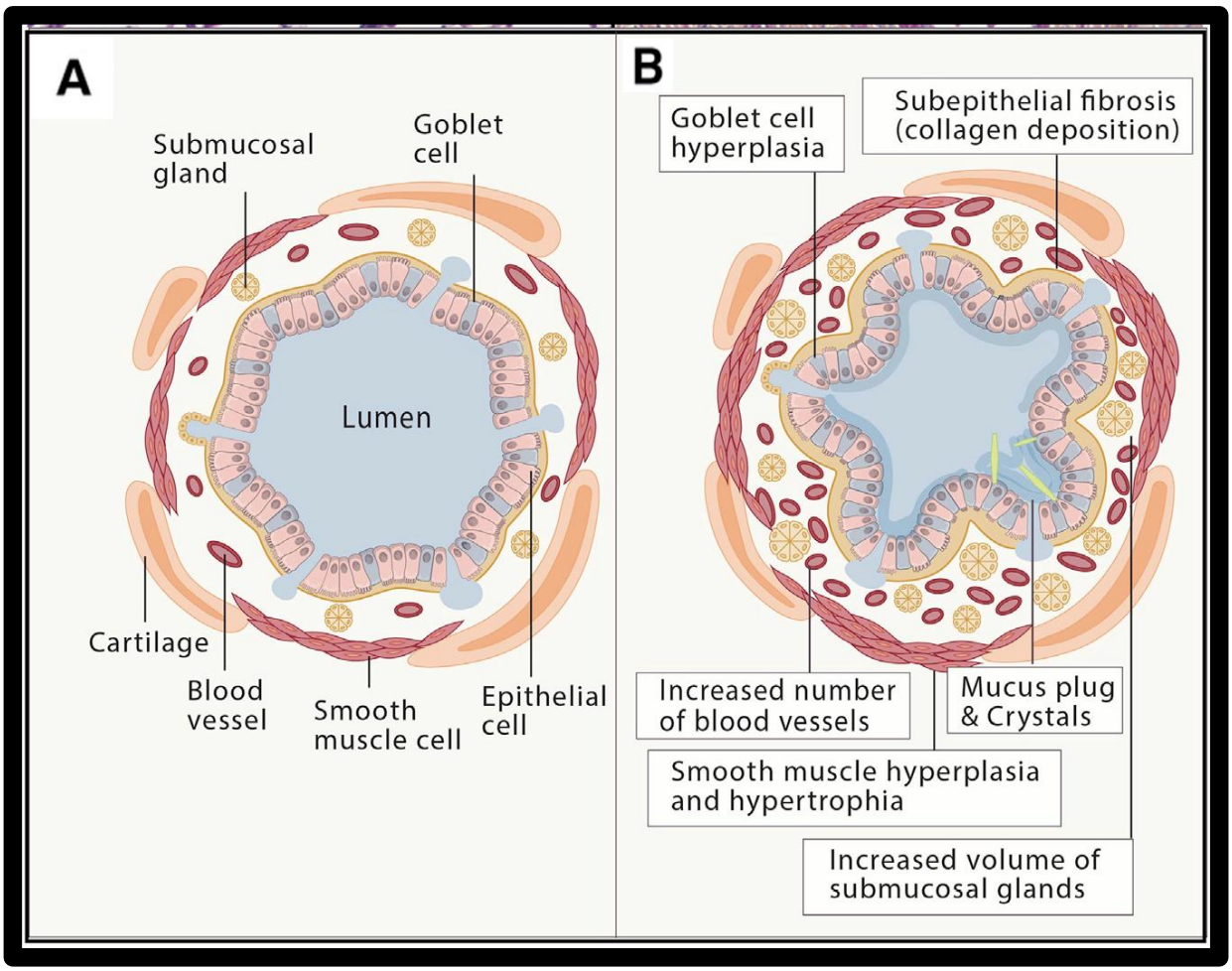

Figure 2.2: Schematic Representation of Differences between healthy (A) and asthmatic lungs (B). (Lambrecht et al., 2019).

2.3. House Dust Mites

House dust mites (HDM) are Arthropods which are animals characterized by external skeletons and limbs, the chelated subtypes as well as arachnids, mites, and astigmatisms are all included in house dust mites animals which also characterized by no-special respiratory-organs) (L. G. Arlian & Platts-Mills, 2001). HDM are not belonging to insects

but belonging to members of the Uniramia class, contrary to popular belief. House dust mites have a totally distinct shape and physiology than insects. As a result, standard pesticides have been effective in destroying the insects. Dust mite populations cannot be controlled by insects. (L. G. Arlian & Platts-Mills, 2001). These bacteria range in size from 0.1 to 0.6 mm and are invisible to the naked eye. A magnifying lens with a magnification of at least ten times is required to appropriately identify them.

Their bodies are round and feature eight feet, ranging in color from cream to translucent white. When males and females mate, house dust mites reproduce sexually. Dust mites begin their life cycle when a fertilized female lays a few eggs per day. The hexapod larva hatches from the egg and continues active for the rest of its life. They removed their coverings and transformed into eight-legged immobile protozoa for a short time. Protonymphs shed their shells and mature into larger, more active triton bugs (Vona, 1997). The length of this development cycle is unknown, however it could last anywhere about two to six weeks in each cycle. Number of eggs of female unknown, however it is thought that she can lay 40 to 100 eggs in her six-week existence. It can live and survive for about two to five ;depending on the climatic conditions; months.

These tiny organisms mostly feed on human and pet dander, which is inhabited by fungus, yeasts, and bacteria, but they can also eat other organic waste that has accumulated in the home. (Colloff et al., 1992). Dust mites and fungi have yet to be definitively discovered, however fungi are thought to hasten the expansion of mite populations (Hart, 1998). As a result, treating fungi as a non-biological element in managing home dust mites does not appear to be of much importance, although more research and the possibility of competitive interactions between different house dust mite species are needed. Allergies to these microbes are caused by the metabolites they emit in their feces. The floating dust cloud visible beneath the light when processing bedding and similar products could contain this form of trash. (Hart, 1998).

House dust mites can be found in almost all furniture in homes and workplaces especially the old ones, as well as in items that meet their survival requirements such as woolen products and fabrics for example carpets, curtains, mattresses, pillows, plush toys, and in papers requirements like books. Tropical bromia (Bt) and house dust mites (Dp), for example, are invariably present in every household, especially in places with high relative humidity reach to more than forty-five percent and high temperatures reach to more than sixty fehrinhite to more than eighty fehrinhite. (Hart, 1998). Dust mites get their water by sucking steam from the air around them. As a result, many scientists advise that indoor humidity be kept low to reduce allergy levels in the home.

According to an Indian study, the number of ticks is lower in the summer than in the rainy season and the winter months of September to October. (Tilak, 1989). Extremely high summer temperatures, low relative humidity reach to about under twenty percent, and a lack of raining were also found to be non-favorite factor for mite growth and reproduction

in this study. In most families, the quantity of dust mites on the bed is higher than that on the floor, which suggests that this may be because these mites are associated to human habitat. They also discovered that older, moist, and poorly ventilated dwellings are better for dust mite survival than new, well-ventilated homes. The severity of allergies in distinct groups of patients with allergies and asthma assessed at the same time is directly proportional to seasonal changes, house dust mite concentrations, and apartment kinds.

Researchers discovered changeing in airway -allergies and other types of parameters were connected to allergen exposure and changes in seasons in a 1997 study of asthma patients sensitized by house dust mites (Van der et al., 1997). They discovered that house dust mite allergen levels were higher in the autumn than in the spring. Another intriguing discovery is hypersensitivity to the airways and total serum of sensitized individuals, as well as specific IgE to house dust mite. The serum IgE levels are also higher in the autumn, and humidity levels are higher than in the spring. You can use acaricides to kill dust mites and wash the cloth with very hot water at least once a week to tackle these germs at home.

In addition, there are additional related household products. It's also a good idea to cover your mattresses and pillows with protective covers, and to utilize forced ventilation or air conditioning in your home. The key to decreasing dust mite exposure is to keep relative humidity below 50%. (L. G. Arlian & Platts-Mills, 2001). These and other highly controlled procedures, on the other hand, must be followed consistently and indefinitely, which is a challenging undertaking for most people. Dust mite exposure can be reduced by using special mattress coverings, miticides, and other anti-allergic items. Dust can be costly. Furthermore, it is difficult to locate in the store.

These control approaches should be used in conjunction with one another, as they can have varied outcomes in various families. (Colloff et al., 1992). Controlling these microscopic organisms is also necessary. It has proven to be effective. Scientific study, on the other hand, indicates that asthma sufferers who relocate to higher altitudes or to residences free of dust mites can experience symptoms such as bronchial allergies, which is why some scientists advocate for steps to eliminate house dust mites. As a viable alternative to antihistamines in the treatment of allergic disorders (Van der Heide et al., 1997).

2.3.1. Blomia Tropicalis

Blomiatropicalis (Bt) is a member of the Glyciphagidae family of house dust mites, which have many long back hairs (bristles), no backing, and no anal suction apparatus; their bodies are covered with small nipples (Van Bronswijk, De Cock, & Oshima, 1973). The whole members of the Blomia genus are distinguished from others in the same family by the lack of scales under the metatarsals and the lack of claws found in other genera. There

are also some minor morphological changes between species. This is how they tell Bt from from the rest. (van Hage-Hamsten & Johansson, 1998).

Because they are mostly found in grain and flour in warehouses, stables, hay, and straw, they are known as "store mites" or "storage mites." (Fernández-Caldas, 1997). Storage mites were first studied in the context of occupational allergy illnesses; other Bt and glycolipid mites be considered ubiquitous in houses, and are classed as "ticks" or "domestic ticks." Among the giant house ticks, Bt has the least studied biology and ecology. Dust mites, on the other hand, have been reported to be a significant source of indoor allergies in several studies, particularly in the southern hemisphere (van Hage-Hamsten & Johansson, 1998). Sugar-eating mites, as well as the majority of other well-known house dust mites, discovered firstly in mammals, especially bird nests, and other environments. This result is not surprising, given that humans habitat share common qualities such as heating, humidity, and food, which is making them a good homes for HDMs. (Colloff, 1998).

2.3.2. Dermatophagoides

Dermatophagoides is belonging to the type of house dust mite species “ which called Pyroglyphidae” that found in tropical climates across the world. (Platts-Mills, 1992). The presence of anal suckers is a distinguishing feature of Pyroglyphidae members. The front and back shields, as well as the body, contain variable length stripes and bristle patterns, resembling "fingerprints." (Colloff et al., 1992). Traditional usage of the phrase "house dust mites" refers to Pyroglyphidae family members who reside almost entirely and continuously in housing dust.

The 2nd International Symposium on Ticks and Asthma Allergens (1990) advised that this family as well as Glycyphagidae family ;which Bt belongs to; be referred to as "house ticks." Dust mites (Dp) and dust mites (Df) are the most frequent members of the Pyroglyphidae family globally. Thirteen of the 49 Pyroglyphidae dust mite species are found in indoor dust, whereas the remainder dwell primarily in bird nests or feathers. (Colloff, 1998). The closely related Pyroglyphidae genera Dermatophagoides and Euroglyphus are the most common in many countries all over the world. (Fernández-Caldas, 1997).

2.3.3. House Dust Mite Allergens

The International Federation of Immunological Societies' (WHO) Allergen Nomenclature Subcommitte has produced rules for classifying compounds as allergens. (Larsen & Løwenstein, 1996). Only evaluate allergens with a higher than 5% IgE response rate. Furthermore, allergens can be classed as "main" or "minor" allergens, based on how closely they are linked to a specific IgE. The subject being tested has an allergic reaction to the allergen in the system. Of course, several inherent elements in determining the

prevalence of IgE, such as test kit selection, patient selection criteria, geographic location, environmental circumstances, and so on, play a role in determining the prevalence of IgE.

To investigate the sensitizing action of certain substances, the panel recommends using at least 20-30 human sera from those who are really allergic. (Marsh, Goodfriend, King, Løwenstein, & Platts-Mills, 1988). Various scientific research have demonstrated that house dust mite allergen is a major cause of allergies and asthma in several nations throughout the world over the last thirty years. (M. Chapman, Smith, Vailes, & Arruda, 1997).

Many allergens' organic properties and structural properties have been unraveled thanks to developments in molecular biology techniques, even though many allergen studies are still being worked-on or have benn non-completed. Since the organic nature of allergens are not proven to be one of the most important or fundamental allergens in their allergenicity, probably play a role in helping and creating the immunological environment "which will be required for the sensitization to an allergen or increase the protein's cap potential to elicit an IgE antibody response". According to scholars, allergenic chemicals with have enzymatic -activity, like the most common enzymes cysteine proteases, whcih lead for stimulating mucosal surfaces and promote their specific processing. (Travers, Walport, Shlomchik, & Janeway, 1997).

IgE production and enhanced TH2 cell response are the best characteristics allergens of group 1 house dust mites. (M. D. Chapman et al., 2000). Der p 1 has been demonstrated to cleave CD23, a low-affinity IgE receptor, disrupting the negative -feedback control of IgE production mediated by this receptor. (Schulz et al., 1997). However, because numerous allergens from any sources ;especially HDM; have been identified to have different sorts of biological activity, the enzymatic function is not required for triggering the IgE response. Other allergens found in house dust mite proteins are including group-three (Group-3) allergens, group four "Der p 4" allergens which have amylase -activity, and group six "Der p 6" allergens with pancreatic coagulation activity. Lactase-like proteases, group ten and tropomyosin (which are considered structural protein) as a fatty acid and allergen binding protein in groups 2, 5, 7, and 12, all of which have unclear biological functions. (Robinson et al., 1997; Stewart & Robinson, 2003).

Some research talk about allergen sensitizing doses, but the internal allergen and asthma report: the third international symposium report Set the allergen concentration to 2 mg allergen per gram of dust (100 mites per gram). (Platts-Mills, Vervloet, Thomas, Aalberse, & Chapman, 1997). As some investigations have demonstrated, allergen stability can also play a role in allergic reactions when the IgE epitope is splitting. (M. D. Chapman et al., 2000). Allergens differ in structure and are classified into distinct protein families based on their biological roles, implying that allergens may share few or no structural or intrinsic traits that make them allergic. (Bufe, 1998).

Other factors such as genetic predisposition or individual IgE response regulatory deficiencies, hormones, bacterial and viral infections, and other potential adjuvants, and type as well as intensity of exposure cannot be ruled out when it comes to allergen sensitization and allergic reactions (One). Given these implications, recombinant allergens with IgE epitopes removed by site-directed mutagenesis could be useful tools for future study and more effective allergy immunotherapy. (Bufe, 1998).

2.3.4. Physiology of Allergen Production

Egg-laying, lateral sebum secretion (influencing membrane permeability and pheromone secretion), and fecal output are the three ways mites excrete or excrete food (including guanine secretion). Although it has not been shown that lateral gland secretions are a source of allergens, more research into their chemical makeup is needed. One of the most well-known allergens is a protease that may be involved in digestion (Der p I). The final result of purine digestion and excretion is guanine (ticks can be used as a specific marker). More information on the chemistry of nut mite feces in various indoor environments would be helpful. (Chua et al., 1988).

2.3.4.1. Relevance to Controlling Mite Numbers

Several physiological processes of ticks that may be related to control measures have been studied (Nihoul, 1993; Oliveira et al., 2007; Warner et al., 2000), including juvenile hormones, pheromones (fear), and mushroom nutritional disorders. Moreover, X-ray sterilization. These methods have not produced promising results so far (Mueller et al., 2010). Pheromones are very expensive and hormones too, and strategies to stimulate fungal growing can be harmful in terms of increasing the number of fungal allergens.

The use of X-ray sterilized male ticks does not guarantee long-term advantages because ticks have a short lifetime (about 3 months) and frequently emerge in clothes. Because of the fact that mites rely on fungus for reproduction, natamycin (a macrolide fungicide) has been used as an Acaridae. While using pesticides, acaricides, or other avoidance measures, it is critical to keep those techniques in mind and to note possible differences in sensitivity across mite species.

2.3.4.2. Biology

When laboratory data are compared to actual measurements taken in the living room, it is clear that air humidity is generally the limiting factor for mite growth. (Platts-Mills, Hayden, Chapman, & Wilkins, 1987). Most families in affluent nations have at least one habitat where ticks may reproduce due to a lack of food and a warm environment. Mites' primary food source appears to be skin flakes and/or fungus that develop on skin flakes, although they may also feed on a variety of other foods. Although no viral or bacterial illnesses have been examined in hot psyllid ticks, the detrimental consequences of fungal

overgrowth in tick cultures have been documented. Other ticks, such as Cheyletidae or Gamasina, prey on pyroglyphid ticks, however these species are ineffective in controlling pyroglyphid tick populations in the home. (Platts-Mills, Hayden, Chapman, & Wilkins, 1987).

2.3.5. Recombinant and Native House Dust Mite Allergens

An excellent recombinant allergen has several characteristics: An efficient recombinant allergen must be able to bind to IgE, excite specific T cells, and degranulate specific basophils, as well as elicit IgE reactions in laboratory animals and cutaneous reactions in humans, as compared to its native equivalent. (Marsh et al., 1988). Previous research have demonstrated that quantity, fair pricing, and quality remain unaffected. The content of recombinant allergen combinations can be properly determined, and these molecules can be standardized more simply. (M. Chapman et al., 1997).

Recombinant allergens also make it possible to study the specific profile of IgE, leading to the identification of specific allergic components that trigger allergic reactions. This is meaning of the patient's reactivity profile which can be tracked. As a result, the patient will be able to alter his medication based on his allergy profile, as well as track new allergen sensitivity or decrease of reactivity to different allergens. Recombinant allergens also have the advantage of being able to be changed to create derivatives or variations. Allergic activity is reduced. (Kraft et al., 1999). This means that during immunotherapy, the recombinant hypoallergenic derivative is less likely to cause adverse effects or allergies. (M. Chapman et al., 1997).

There's a chance you'll have side effects or allergic reactions. Many recombinant proteins of house dust mite allergens have been made, and the great majority of these react with IgE antibodies in the same way as native allergens do. According to a research, the levels of native RAST, Der f 1 , in addition to the Der f 2 “ also their recombinant analogs roles” are highly correlated. (Noguchi, Shibasaki, Nishiyama, Okumura, & Takita, 1996).

The IgE binding capabilities of recombinant and natural allergens are approximately equal, while native proteins have somewhat greater RAST reactivity than recombinant proteins. Purifying and studying natural allergens in order to compare their biological activity to that of recombinant allergens is, of course, important in order to lay a solid foundation for usage of recombinant allergens which were commonlt mentioned in scientific research and treatment. Natural allergen characteristics, as well as the cloning and expression of their recombinant analogs, should all be included in a holistic approach to house dust mite research. (Pauli, 2000).

In the recent decade, molecular biology techniques have been used to create a large number of recombinant allergens, and the number of accessible recombinant allergens is rising. Allergenic components in a variety of house dust mite species have been

discovered and described as a result of this discovery. These allergens, such as Bt, Dp and Em, have been discovered through molecular cloning of the most prevalent house dust mites. Many of the Dermatophagoides allergens (from the Pyroglyphidae) have been cloned, and a number of them have been produced as recombinant proteins in both bacteria and yeast cells. (Lynch et al., 1997; Kraft et al., 1999).

A single allergen " or named as Eur m 1 from Em was discovered in the early 1990s" resulting in an 85 percent series match with Der p 1. Kent, Hill, Keen, Holland, and Hart (Kent, Hill, Keen, Holland, and Hart, 1992). Despite the fact that most home dust mite allergens have a molecular weight of 10-70 kDa, Mag three with molecular weight of 177 kilodalton. In many cross-reactivity experiments, uncommon allergens such as Dermatophagoides species, Lepidoglyphus destructor (of the Glycyphagidae family) and Bt have been used as the allergens (Smith et al., 2001). Blot five (molecular weight: 14 kDa) from Bt was cloned and generated as a recombinant protein with 43 percent similarity to Der p five (with M.W.: 14 kilodalton) from the Dp.

There were IgE antibodies to the protein in 69% of the sera from bronchial allergy patients, demonstrating that the recombinant protein was a significant allergen. For individuals with bronchial allergies who live in tropical climates, Bt allergens are a different source of sensitization, according to the researchers. (Arruda et al., 1997).

The discovery was made by Caraballo and his colleagues in 1998 that Bt-M is the C-terminal section of Blo t five and has a lot of cross-reactivity with Der p 5. IgE-binding was found in 50% of Bt11a, suggesting it is an allergen of vital importance, according to Puerta and colleagues. In the case of Caraballo and colleagues, this involved subcloning and sequencing of a Bt6-cDNA. To validate 11 percent IgE reactivity, the 130 amino acid protein of 14.8 kDa was tested on allergy sufferers' sera. (Puerta et al., 1996; Caraballo et al., 1997; Caraballo et al., 1998) .

Blot 13 was given to Bt6 after it was better described. This allergy shares a sequence with the cytosolic lipid transfer protein (cLTP) family, making it the first tick-borne allergen to have a fatty acid pooling activity. Other tick species are expected to have homologs to Blo t13 because to the highly conserved structure and essential biological functions of cLTPs; due to the highly conserved structure and important biological functions of cLTPs, other tick species are likely to have homologs to Blo t13. (Puerta, Kennedy, Jiménez, & Caraballo, 1999).

Yi and colleagues described the identification and sequencing of Blot 10, a novel Bt allergen. Tropomyosin is the allergen in question, and its amino acid identity with a set of ten mite allergens (such as Der p 10) is as high as 96%. Tropomyosin is a muscle protein found in various invertebrate species and is a key allergenic component. Blot 10 and Der p 10 were also studied, and the researchers discovered that, despite being very conservative and showing considerable cross-reactivity with each other, they contain

distinct IgE epitopes. Both allergens must be taken into consideration in the diagnosis of home dust- mite sesitization in locations where these two species of house dust mites are predominant. (Yi et al., 2002). Recombinant Bt allergens from group 1 called Blot 1 were cloned and expressed (Mora, Flores, Montealegre, & Diaz, 2003).

House dust mite cysteine protease Der p 1, Der f 1, and Eur m 1 are also 35 percent identical. Tests of this recombinant Bt protein with serum from Bt-positive patients revealed IgE reactivity of 62%. Bt hypersensitivity can be diagnosed by testing for rBlot t 1, which is the most common allergen found in house dust mites. (Ramos, Cheong, Lee, & Chua, 2001).

It was recently reported that Bt cDNA corresponding to group 3 house dust mite allergens was cloned and molecularly characterized. The Bt2-3 clone encodes a 231-amino-acid-residue mature protein with a molecular weight of 27.5 kDa and shows high sequence similarity (48-54%) with other tick-derived serine proteases. (Protease that is similar to trypsin). Imprinted 11, a Bt allergen with similarity to numerous invertebrate paramyosins, is another Bt allergen. These patients, who are seropositive in 52 percent of asthma patients, have potentially substantial allergic activity. (Ramos, Cheong, Lee, & Chua, 2001).

2.3.6. Treatment of House Dust Mite Allergy

Scientists have been studying innovative strategies to cure HDM- allergy for the previous ten to fifteen years. Allergy symptoms can be reduced using a variety of medications. These medications, on the other hand, must be used indefinitely. Many of them have drowsiness as a side effect “ which can impair the patient's daily performance”, other drugs that are effecting more and do not cause drowsiness should be prescribed, even if they are more expensive; home prevention and control measures must be strictly followed, and these methods can be implemented. Another popular preventive treatment is the use of house dust mite allergens for immunotherapy or desensitization. The patient has been injected for one to four years, with the dose of mite gradually increasing until reach to the maintenance dose is attained, starting by once in week and gradually decreasing to once or twice a month depending on the patient's reaction. (Vona, 1997) .

The procedure's goal is to It gradually increases the patient's immune system's tolerance to mite allergens. Immunotherapy is a successful treatment, but scientific study demonstrates that the specific mechanism by which it works is yet unknown. The total production of particular inflammatory cytokine allergens including IL-4, IL-5, and interferon-gamma (IFN-g) is reduced after immunotherapy. (O'brien, Byron, Varigos, & Thomas, 1997). Allergic immune response to TH1-like responses (i.e. immunological interference), lack of peripheral T cell response (energy), or possible absence of allergen-reactive lymphocytes are all examples of TH2-like reactions. Effective, but has the advantage of addressing allergic causes rather than merely symptoms, resulting in allergic

reactions being eliminated or considerably reduced. Immunotherapy is the best treatment techniques for house dust mite allergies, according to extensive study. However, it has been discovered that using house dust mite extract has a number of drawbacks, including the risk of allergic reactions (a severe allergy) (Response disease). Life-threatening allergic reactions, even if very low dosages appear to be diminished with time; also, standardizing the balance of allergens and non-allergenic substances in mite extracts, such as protein, carbohydrates, and nucleic acids, is problematic. (Valenta & Vrtala, 1999).

The extraction procedure, storage conditions, and some allergies all have an impact on the quality of the extract. It's possible that it won't be adequately represented in mite extracts, and that it'll even be degraded. (Valenta, Vrtala, Laffer, Spitzauer, & Kraft, 1998). The extract can help you identify the allergen's source, but it can't tell you which molecules the patient is allergic to or what level of IgE they have for that allergy. The usage of original mite extracts is primarily used in the present diagnosis and treatment of house dust mite allergies. Diagnosing and developing treatments based on reactive properties is a more successful strategy. This technique can detect allergen components, particularly mites. The disease that causes the disease and the IgE level determination for these disorders. The design and availability of recombinant allergens are critical in this scenario.

2.4. Dust Mites Induced Asthma

House dust mites are the leading cause of atopic sensitization and allergy disease worldwide. Dust mites that are the most allergic are: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae, Euroglyphus maynei,* and *Blomia tropicalis* are eight-legged members of the Arachnid class. The egg, larval, protonymph, tritonymph, and adult stages of their approximately 3-month existence are visible, with adults measuring around one fourth to one third of a millimeter in size. Dust mites' regional and seasonal distributions are influenced by their need for proper humidity, while their dislike of light influences their dispersion within substrates. (Miller, 2018).

By contacting the epithelium of the eyes, nose, lower airways, skin, and stomach, dust mite allergen-containing particles can produce sensitization and atopic symptoms in multiple organs. Clinical problems caused by mite sensitization and exposure include rhinitis, sinusitis, conjunctivitis, asthma, and atopic dermatitis. Ingestion of cross-reacting crustaceans like shrimp or snails, or unintended ingestion of mite-contaminated foods, can also trigger systemic allergy reactions. In addition to their immediate role as a key allergen source, dust mites provide insight into the nature of atopy and allergy sensitivity in general (Miller, 2018).

Recent years have seen extensive investigation into the link between HDM allergens, the host immune system and airways. Dermatophagoides pteronyssinus aeroallergen

hypersensitivity is responsible for 50–85 percent of asthmatic atopic sensitization. (Sanchez-Borges et al., 2017; Calderon et al., 2015; Gregory et al., 2011).

Mite sensitization and exposure, in addition to genetic predisposition, are key predictors of asthma development. As a result of exposure to mite allergen, patients with asthma and dust mite sensitivity have exacerbated bronchospasm and bronchial hyperreactivity, whereas symptoms are alleviated in a mite-free environment. (Platts-Mills and Chapman, 1987; Arlian and Platts-Mills, 2001; Thomas, 2012).

The fact that HDM exposure and sensitization levels are good predictors of asthma in clinics further supports the link between HDM and asthma. (De Alba et al., 2010; Birrell et al., 2010). In addition, the interaction of mite sensitization, exposure, and respiratory virus infection worsens the condition and is the major cause of acute wheezing and hospitalization. (Soto-Quiros et al., 2012; Murray et al., 2006; Green et al., 2002). Other evidence linking HDM and asthma includes the fact that inhaling HDM allergens causes bronchial smooth muscle proliferation and the detection of HDM-specific IgE in asthmatics' sputum. (Trian et al., 2015; Mouthuy et al., 2011).

HDMs are defined by protease activity, immunogenicity, and stimulation of the innate immune system, despite the fact that only four of the more than 30 allergens found in House Dust Mites are proteases. (Jacquet et al., 2020). Lipopolysaccharide (LPS),-glucan, and chitin are also components of HDMs. (Gregory et al., 2011). Various components of HDMs appear to trigger the immune system, based on the above. The allergenic effects of HDMs have been identified thanks to recent technical breakthroughs. Proteolysis and peptide-lipid/lipid binding, two biological actions of allergens, produce IgE and trigger bystander responses to unrelated allergens. (Jacquet et al., 2020). (Figure 2.3).

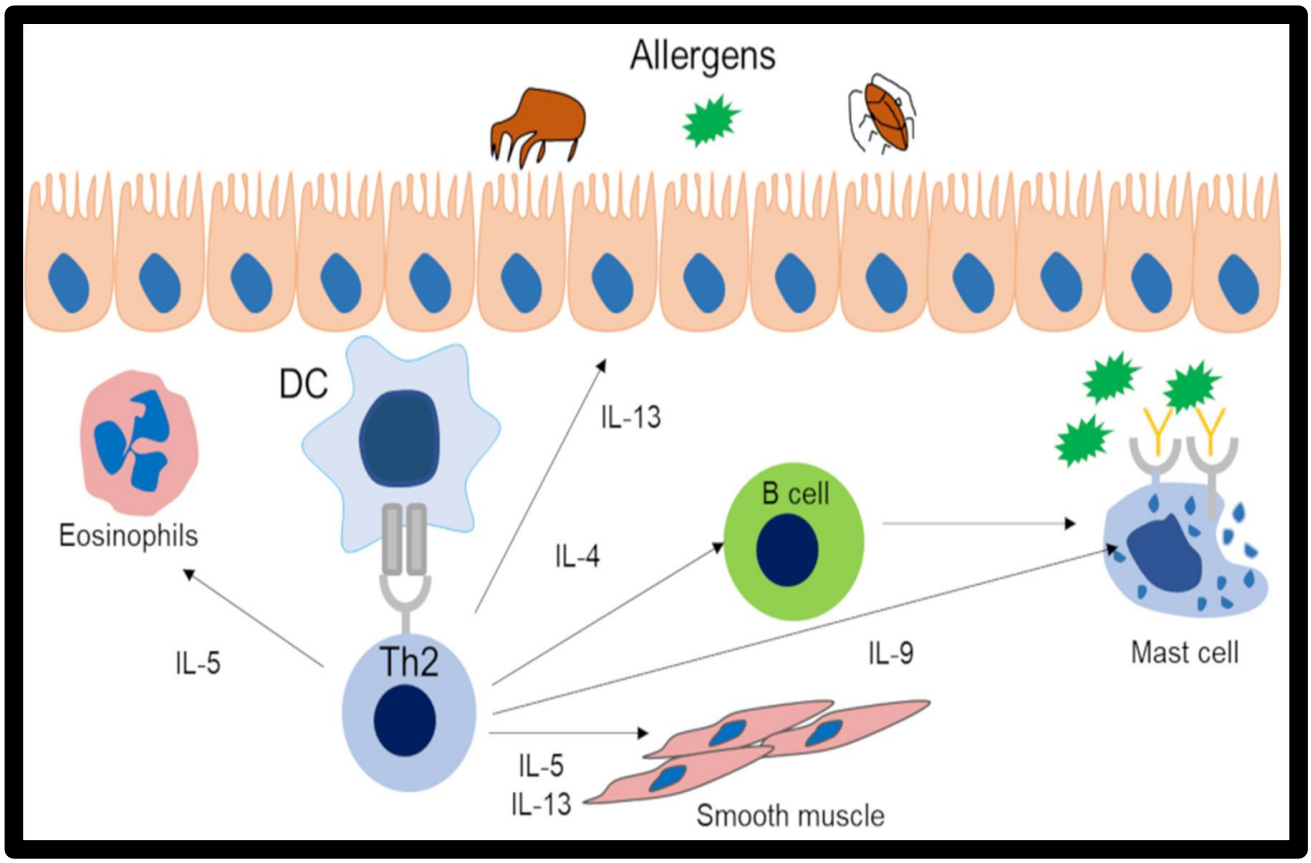

Figure 2.3: Type 2 immune response via Th2 lymphocytes in asthma patients. (Yasuda et al., 2020).

2.5. Role of Cytokines in Pathophysiology of Asthma

Pleiotropic cytokines are a group of new mediators that govern asthma's intermittent airway inflammation, bronchial smooth muscle hyperreactivity, and broncho-constriction. The pathophysiological aspects of asthma are hypothesized to come from the abnormal proliferation of cytokines and chemokines, according to preclinical and clinical findings. Cytokines are tiny extracellular signaling proteins produced by a variety of cells, and their redundancy and pleiotropic qualities allow them to perform a wide range of biological functions. Both innate and adaptive immune responses rely on them to function properly. Cytokines and their receptors have a strong attraction to one another. Picomolar quantities of cytokines can have a biological effect due to their high affinity. Lymphokines, proinflammatory cytokines, inhibitory cytokines, and growth factors are the four major types of cytokines. Asthma's pathophysiology is still poorly understood, and the cause is unknown. This review will focus on the production, release, and functional significance of each cytokine in the pathophysiology of asthma based on this foundation. (Mahajan and Mehta, 2006).

2.5.1. Interleukin 13

Clinical and physiologic studies have connected asthma to edema-induced airway constriction and the infiltration of numerous inflammatory cells into the airway lumen wall. These cells include eosinophils, mast cells, IgE-producing plasma cells, and a subset of activated T cells known as Th2 lymphocytes. In spite of the fact that the precise physiological handle fundamental these cellular reactions is obscure, complicated interactions between these cells within the aviation route in reaction to antigen come full circle within the creation of cytokines and other provocative proteins, which make chemical go between pivotal in this condition. IgE production, goblet cell hyperplasia, mucus hypersecretion, airway hyperresponsiveness, fibrosis, and chitinase up-regulation are all regulated by interleukin-13. It has a role in allergic inflammation and a variety of illnesses, including asthma. (Rael & Lockey, 2011).

2.5.1.1. The Role of Interleukin 13 in Asthma

IL-13 is to a great extent connected to the advancement of aviation route ailment, in spite of the fact that it too has anti-inflammatory impacts. Within the aviation routes, IL-13 enacts a family of protein-degrading chemicals known as network metalloproteinases (MMPs). These proteins are fundamental to cause parenchymal fiery cells to attack the aviation route lumen, where they are evacuated. IL-13, among other things, produces these MMPs as portion of a defensive instrument against extreme unfavorably susceptible irritation, which can lead to suffocation. (Minty et al., 1993).

Eosinophil survival, activation, and recruitment have all been linked to interleukin-13. In vitro cultures of eosinophils treated with recombinant IL-13 demonstrated a dose-dependent increase in survival, which was attributed to apoptosis inhibition. Eosinophils stimulated or released IL-3 and GM-CSF, which was mediated by an autocrine pathway. In the asthmatic airway, one of the main functions of IL-13 (and IL-4) is to cause eosinophil chemotaxis to the site of injury. IL-13-induced chemotaxis and eosinophil activation have been studied in vitro in several investigations. (Rael & Lockey, 2011). (Figure 2.4).

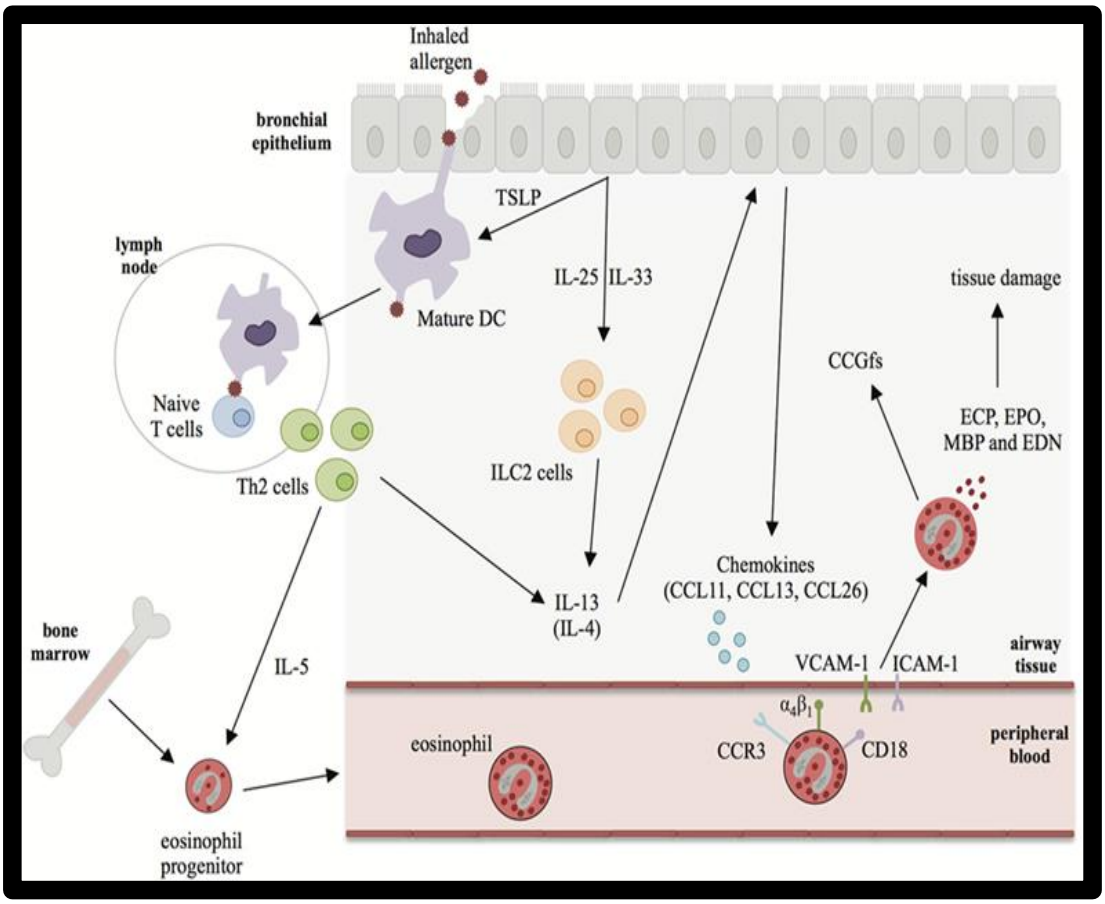

Figure 2.4: The role of interleukin (IL)-13 in driving eosinophilia in asthma. (Doran et al., 2017).

2.5.2. Interleukin 9

Interleukin 9 (IL-9) is a pleiotropic cytokine (cell signaling molecule) that belongs to the interleukin family. IL-9 is generated in varying levels by a number of cells, including mast cells, NKT cells, Th2, Th17, Treg, ILC2, and Th9 cells. Th9 cells are the most common CD4+ T cells that generate IL-9 among them. (Kaplan & Perumal, 2011).

2.5.2.1. The Role of Interleukin 9 In Asthma

Edema-induced airway constriction and the infiltration of many inflammatory cells into the airway lumen wall have been linked to asthma in clinical and physiologic studies. Eosinophils, mast cells, IgE-producing plasma cells, and a subgroup of activated T cells known as Th2 lymphocytes are among these cells. Despite the fact that the precise physiological handle fundamental these cellular reactions is unknown, complex interactions between these cells within the aviation route in response to antigen come full circle within the creation of cytokines and other provocative proteins, making chemical communication crucial in this situation (Holgate et al., 1995). Despite these

advancements, the etiology of atopic asthma remains a mystery. Susceptibility to atopic asthma is most likely multigenic. The importance of IgE and other cytokines in the allergic and inflammatory responses, as well as biologic variability in the allergic and inflammatory responses, has motivated research into potential genes for structural and functional genetic variability. On chromosome 5q31-q33 in humans, a major asthma gene was recently identified. (Banks-Schlegle 1997, Postma et al., 1995).

Although the gene (s) responsible for asthma, airway hyperresponsiveness and allergies have not yet been discovered, 5q31q33 is known to be a fusion gene or to share a chromosomal structure with regions of chromosomes 11, 13 and 18 of the mouse.(DeBry & Seldin, 1996). Various quality candidates, such as cytokines, development components, and development calculate receptors, are found on chromosome 5q31-q33, which may have a part within the aviation route aggravation related with atopic asthma. Based on linkage disequilibrium between log serum add up to IgE levels and a marker interior this quality, interleukin 9 (IL-9) was proposed as a conceivable candidate.(Doull et al., 1996).

A subjective locus (called qualitative trait locus QTL) that maps to the syntenic location of chromosome 13 appears to have a role in determining important differences in bronchial responsiveness between hyporesponsive C57BLy6J (B6) and hyperresponsive DBAy2J (D2) mice. Bronchial hyporesponsiveness is linked to a considerably lower steady-state of IL-9 levels, which is linked to a genetic change at the B6 locus, according to the findings of a test examination that looked at IL-9 as a quality candidate.

This was reflected in decreased levels of this cytokine in the lungs of B6 mice compared to the lungs of bronchial hyperresponsive D2 animals. These findings suggest to IL-9 as a viable candidate in the complex etiology of bronchial hyperresponsiveness, a critical component of the asthmatic response. Figure 2.5 depicts the regulating role of IL-9 in inflammatory cells associated with allergy disorders.

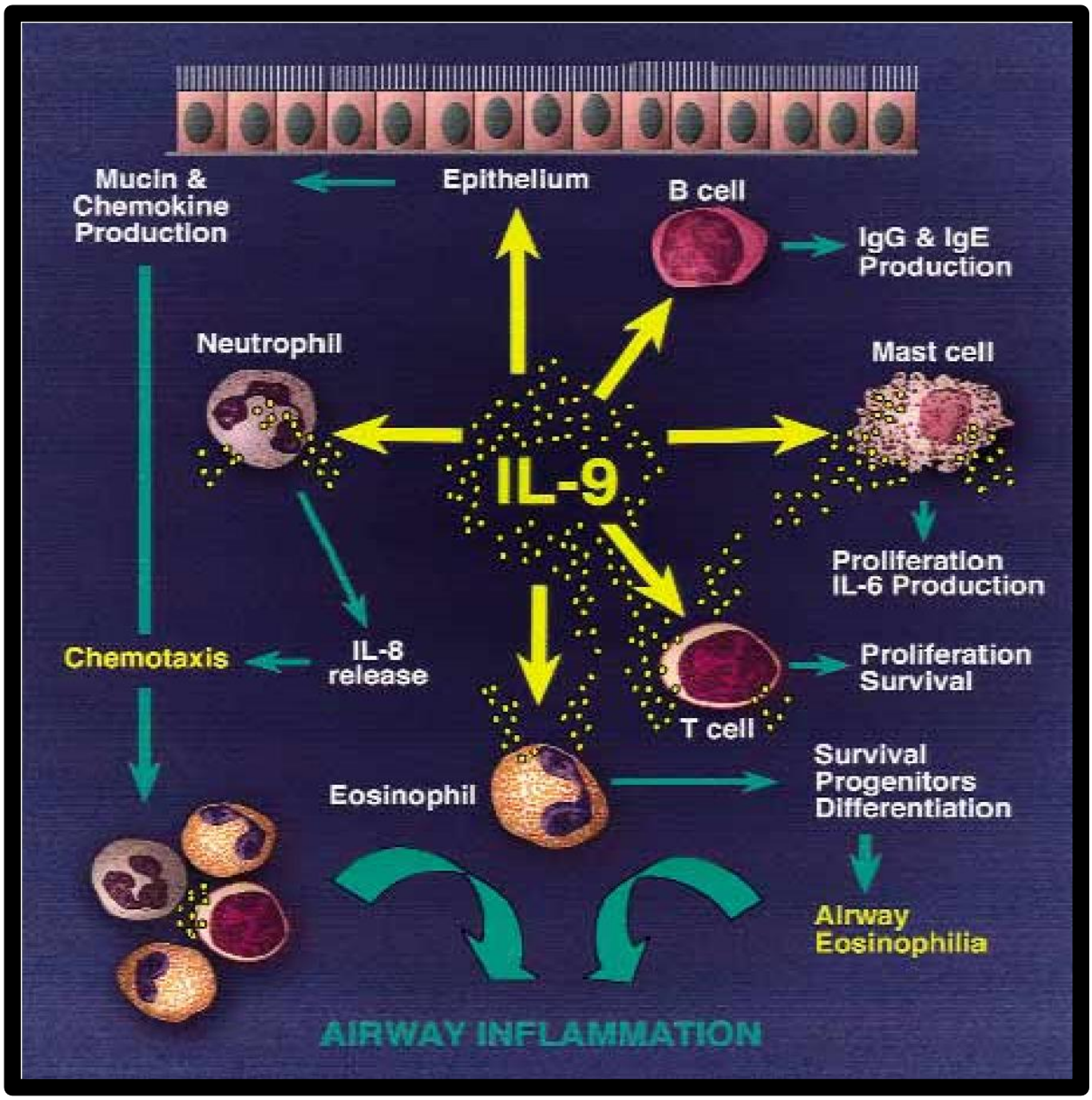

Figure 2.5: Potential role of IL-9 during allergic inflammation in the airways. (Soussi-Gounni et al., 2001).

2.5.3. Tumor Necrosis Factor TNF

TNF is one of the multiple proteins capable of inducing necrosis (death) of tumor cells that possess a wide range of proinflammatory actions. (Akoğlu, Babayiğit, Karazincir, Balcı, & Hanta, 2008). TNF could be a multifunctional cytokine with impacts on lipid digestion system, coagulation, affront resistance, and the work of endothelial cells lining

blood vessels. TNF is utilized by the safe framework for cell signaling. In case macrophages identify an contamination, they discharge TNF to caution other resistant framework cells as part of an fiery reaction. TNF could be a part of the TNF superfamily, which comprises of different transmembrane proteins with a homologous TNF space. (Heir & Stellwagen, 2020).

2.5.3.1. Association of Tumor Necrosis Factor with Asthma

Asthma is considered to be a sort 2 (Th2) T aide infection with a clear cytokine discharge profile, counting interleukin 4 (IL4) and interleukin 5 (IL5). Be that as it may, increasingly prove appears that other cytokines customarily considered to have a place to the Th1 sort range are moreover related to the incendiary reaction characteristics of human asthma. One of these go between is TNF-a, which is related to asthma irritation due to different in vitro, in vitro, in vivo, and hereditary considers. It has been appeared to extend aviation route hyperresponsiveness (Thomas, Yates, & Barnes, 1995).

This extreme touchiness response of aviation route smooth muscle may be related to the enrollment of incendiary cells, coordinate impacts on aviation route smooth muscle, or the improvement of a arrangement of incendiary responses taking after the discharge of go between, counting due to expanded histamine levels Distribution. (Anticevich, Hughes, Black, & Armour, 1995; Shah, Church, & Holgate, 1995). It is of one of a kind leisure activity that TNFa too can reason a boom in simple muscle eotaxin innovation and emission (at the side of IL1B) from the human aviation route simple muscle, with eotaxin being essentially tried within the asthmatic aviation route muscle. TNFa may have extra backhanded remodeling action since it can initiate the discharge of network metalloproteinases (MMP) from eosinophils and fortify the union of glycosaminoglycans in human lung fibroblasts (Elias, Krol, Freundlich, & Sampson, 1988).

These data show the potential multiple functions that TNFA can perform. Play a role in asthma, increase smooth muscle sensitivity, activate myofibroblasts and fibroblasts, and use IL-4 and IL-5 to regulate the activity of eosinophils. Neutrophils play an increasingly important role in the pathogenesis of asthma. The neutrophils of asthma patients show enhanced migration response, which increases the production of superoxide and its secretion products, thereby The contractility of the bronchial ring confirms this. (Håkansson, Carlson, Stålenheim, & Venge, 1990).

A few autonomous ponders on TNF polymorphisms have appeared that it is related to asthma, with specific accentuation on TNFa-308G>A, due to its known association in differential translation activators, raised plasma TNFa levels, and higher levels of TNF Within the body. In vivo and in vitro incitement (Wilson, Symons, McDowell, McDevitt, & Duff, 1997). It has been reported to be associated with an increased risk of asthma (12, 14, 29-31), atopic (Lin et al., 2002), and bronchial hypersensitivity (Moffatt & Cookson, 1997). On the other hand, several negative correlations between the TNFA-308G>A and

asthma and/or asthma-related phenotypes have also been published (Lin et al., 2002). The frequency of secondary alleles (TNFA-308A) in asthma patients is higher than that in the control group. However, a study also reported the opposite result (the incidence of asthma is lower in patients) (Moffatt & Cookson, 1997). Several independent studies have shown that TNFA-308G> A promoter polymorphism is associated with asthma risk (Albuquerque et al., 1998). Stallions show no significant association, and the polymorphism of the TNFB-252A>G intron is associated with asthma (Albuquerque et al., 1998). (Figure 2.6).

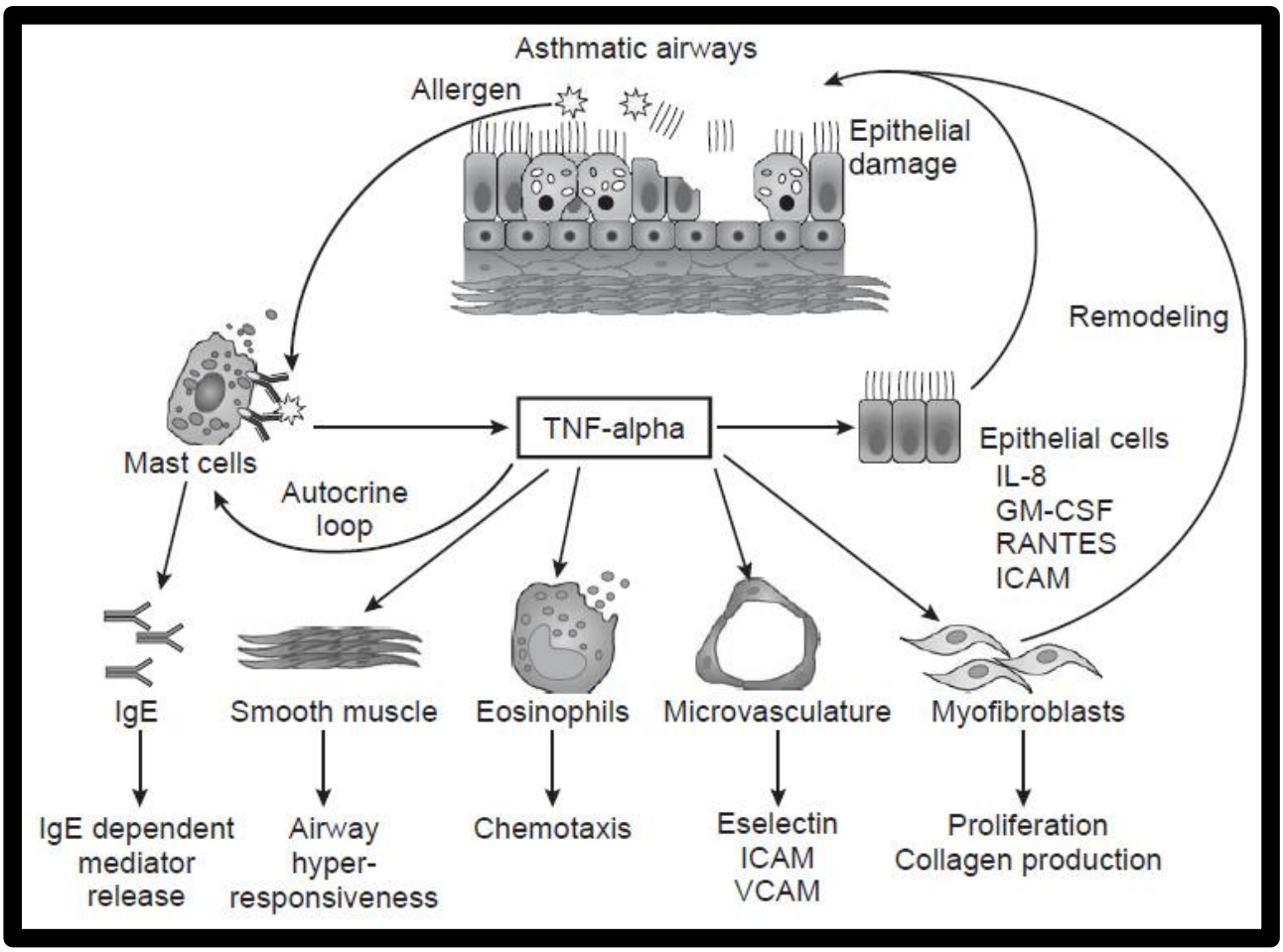

Figure 2.6: Mast cells are the major source of TNF-a, and this release usually is mediated by allergen cross-linking of the IgE molecules. (Babu et al., 2004).

3. MATERIALS AND METHODS

3.1. Study Design

After obtaining the approval of the Ethics Committee, a cross-sectional observational study will be conducted in the Allergy Specialization Center (Al resafa, Baghdad, Iraq) with signs and symptoms suggestive of mite allergy to determine the allergen profile in Iraqi patients.

3.2. Collection of Samples

100 patients (age: 18-40 years) with mite allergic will be selected after careful screening, and the control group consists of 50 healthy participants taking into account the same age group. Patients will be examined and diagnosed at the clinic, information on age, gender, and allergen type and source will be recorded in the data form. The diagnosis of mite allergic will be based on the guidelines for (Mite allergic and their effects on asthma) and GINA (Global Asthma Initiative), respectively.

3.2.1. Selection of Patients

The experiments carried out in the application part of this study were carried out in the Allergy Expertise Center Laboratory in Baghdad, Iraq. Directed by the Internal Diseases and Ear Buran Throat Clinics to Allergy Specialist Center Allergy and Immunology Clinic; Patients with complaints of runny nose, nasal obstruction, sneezing, and sneezing were included in the study with their consent.

3.2.2. Questionnaire

Several criteria have been taken into consideration in forming the control and experimental groups.

The conditions observed in forming the experimental group are:

-Being over 18 years old.

-Not having a disease, such as upper or lower respiratory tract infection, that/ would affect the study results and method application in the last three months.

-Not being treated for any cancer, not being involved in treatment such as chemotherapy or radiotherapy.

-May have an impact on laboratory diagnosis results; Not being diagnosed with metabolic syndrome, Type - 1 and/or Type - 2 Diabetes.

There are no factors that negatively affect work such as obesity, smoking, drug abuse.-

-It is not possible to use any drugs, especially antihistamine drugs, that may affect the study.

The conditions observed in forming the control group are:

-Not having a disease, such as upper or lower respiratory tract infection, that would affect the study results and method application in the last three months

-Not being treated for any cancer, not being involved in treatment such as chemotherapy or radiotherapy.

-May have an impact on laboratory diagnosis results; Not being diagnosed with metabolic syndrome, Type - 1 and/or Type - 2 Diabetes.

-There are no factors that negatively affect work such as obesity, smoking, drug abuse.

-No use of antihistamines or any other drugs.

-Lack of diagnosis of mite allergic (This control and examination were performed by the specialist physician in the clinic and the health status of each participant was approved by the physician).

Verbal and written information about our study and experimental practices were given to 100 patients in the experimental group and 50 patients in the control group, questions of each patient and participant were answered, consent forms were signed and their consent

was obtained. However, approval was obtained from the Chief Physician Board and the Committee.

Each patient and participant constituting the experimental and control groups were interviewed under the supervision of a specialist physician and special information was obtained on some issues. In this context, information was received on the following points and the answers were noted:

-Whether there is a family history of allergies and allergic rhinitis.

-The nature of the allergic rhinitis complaint.

-Disease history, occurrence, frequency, and recurrence of the disease.

-The course of the disease, the factors that trigger the disease.

-Smoking, alcohol, and drug abuse.

-Pet feeding status.

-Questions about cleaning (personal and house cleaning).

3.2.3. Collection of Specimens

Under aseptic conditions, a total of 10 ml blood samples were taken from each patient and control into vacutainer tubes and were leaved for 30 minutes for spontaneous clotting at room temperature before being centrifuged at 2500 rpm for 5 minutes to separate serum. Serum samples were used for estimation of total IgE, specific IgE, IL-13, IL-9 and TNF-α.

3.3. Methods

3.3.1. Estiamtion of Total IgE

Total serum IgE was determined by immune-enzymetric assay using the total IgE ELISA kit (Euroimmum/German). Table 3-1 shows the chemical reagents that found in this kit.

Table 3.1: Contents of total IgE kit.

Reagents	Components
Antibody-coated microplate	96 well plate
Calibrator 1	500 IU/ml (IgE, human), ready to use
Calibrator 2	100 IU/ml (IgE, human), ready to use
Calibrator 3	10 IU/ml (IgE, human), ready to use
Calibrator 4	0 IU/ml (IgE, human), ready to use
Positive control 1	(High IgE concentration) ready to use
Positive control 2	(Low IgE concentration) ready to use
Enzyme conjugate	Peroxidase-labelled anti-human IgE (mouse), ready to use
Diluent buffer	Ready to use
Wash buffer	10X concentrated
Chromogen/substrate solution	TMB/H2O2, ready to use
Stop Solution	0.5 M sulphuric acid, ready to use

- Procedure

1- All sera were diluted by sample diluent buffer (100µl serum to 900µl diluent). Then 100 µl of calibrators, positive controls, and diluted samples were transferred to wells, at room-temperature the plate was incubated for 30 minutes.

2- After incubation, the plate was washed three-times by buffer solution, then 100 µl enzyme conjugate was added for each well, the incubation at room-temperature for thirty minutes, after that washing step by ELISA washer.

3- Substrate/chromogen (100 µl) was added to each well, the plate was incubated at room temperature for 15 minutes. Then 100 µl of stop solution was added to each well.

4- Photometric analysis of the colour-intensity was made at a wave-length of 450 nm and a reference wave-length between 620 and 650 nm, after 5 minutes of adding stop solution.

- Calculation and Interpretation of Total IgE

The concentration of antibody was estimated by plotting the absorbance of standards on Y axis and the concentrations (IU/ml) on X axis on same graph curve. From the standard curve the concentrations of tested samples were calculated. Figure 3-1 is illustrated the standard curve of total IgE.

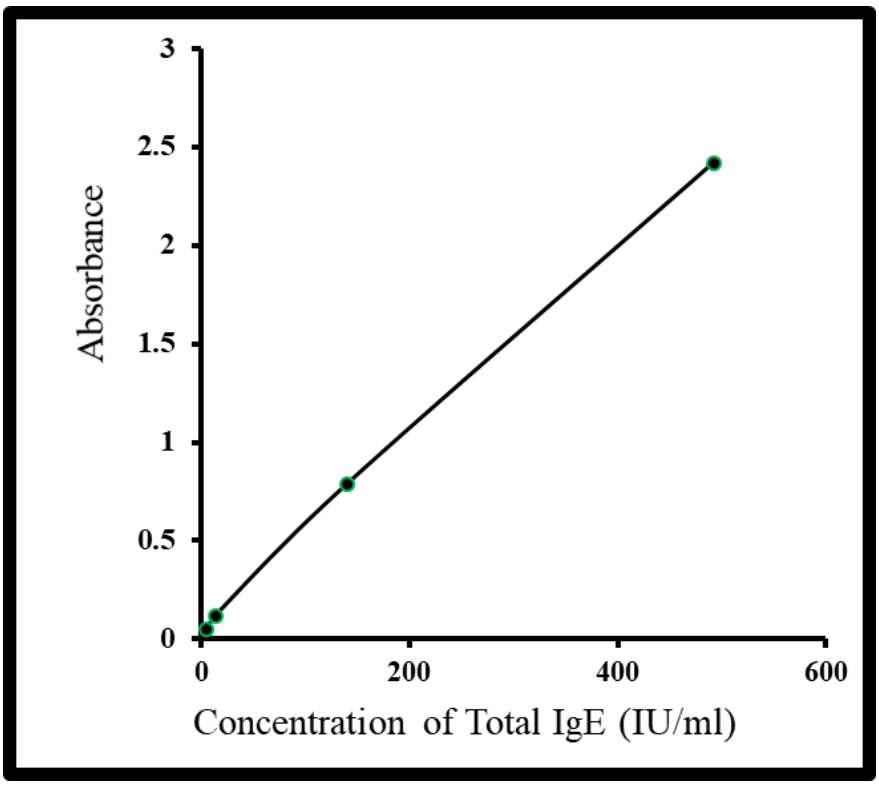

Figure 3.1: Cubic standard curve of total IgE (IU/ml).

3.3.2. Determination of Specific IgE

Determination of mite allergen specific IgE in serum of total subjects was estimated by using (Polycheck-Allergy Diagnostic/Germany) kit which is containing twenty of common inhaled allergens. Table 3-2 lists the chemical reagents of the kit.

Table 3.2: Contents of Specific IgE kit.

Reagents	Components
Start solution	Buffered protein solution
Anti-IgE antibody	Monoclonal (murine) antibody labelled with ligand
Enzyme-labelled anti-ligand	Ligand conjugated to alkaline phosphatase
Substrate solution	5'bromo-4'chloro-3'indolylphosphate and 4'nitroblue tetrazolium, buffered
Wash buffer	Phosphate buffer, pH 7.4

- Procedure

1- The Polycheck allergy cassettes were moisturised with 1 ml wash buffer, then the washed buffer was removed by tapping the cassettes upside-down on absorbent paper. Then allergy cassettes were overlaid with 250 µl of Polycheck start solution and incubate for 60 seconds.

3- A 200 µl of the respective patient's serum was added into the cassette and incubate for 60 minutes on a shaker.

4- By 1 ml of Polycheck wash buffer, the cassettes were decanted and washed three times, the cassettes were tapped carefully upside-down on absorbent paper. Wash buffer (250 µl) was added and incubation for 5 minutes on a shaker. Then washing was repeated.

5- Polycheck anti-IgE antibody (250 µl) was pipetted, then incubation for 45 minutes on a shaker. The cassettes were decanted and washed three times with 1 ml washing buffer.

6- Polycheck enzyme-labelled anti-ligand (250 µl) was added, then incubation for 20 minutes in the dark. The cassettes were decanted and washed as in previous step.

7- polycheck substrate solution (250 µl) was pipetted, and the cassettes were incubated for 20 minutes in the dark. After that the cassettes were decanted and washed as in previous step. The membrane was air-dried and the Polycheck allergy cassettes were evaluated by using a scanner and the Biocheck Imaging Software.

3.3.3. IL-13 Levels

Serum levels of IL-13 were estimated by using Bioassay/China ELISA kit in asthmatic patients with mite allergy. Table 3-3 lists the chemical reagents of the kit.

Table 3.3: lists the chemical reagents of the IL-13 kit.

Components	Quantity
Standard Solution (6400pg/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IL-13Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

- Procedure

1- The reagents which incorporate standard arrangements, and tests were arranged concurring to the informational parts. Some time recently utilize, the total reagents were brought to room temperature. The try was carried out at room -temperature.

2- For the test, the number of strips vital was set up. For utilization, the strips were put into the outlines. The unused strips ought to be kept at a temperature of 2-8°C.3- Standard

was added to the standard well in the amount of 50 microliters. (Note: Because the standard solution contains biotinylated antibody, the antibody did not contribute to the standard well.)

4- 40 microliter of sample was included to test wells, taken after by 10 microliter of anti-interleukin 13 counter acting agent, 50 microliters of streptavidin-HRP, and at last 50 l of streptavidin-HRP to standard wells (Not clear control well). The plate was well blended, at that point fixed with a sealer and hatched at 37°C for 60 minutes.

5- After expelling the sealer, the plate was washed five times with wash buffer. For each wash, wells were splashed for 30 seconds to 1 diminutive with at slightest 0.35 ml wash buffer. All wells were suctioned and washed five times with wash buffer some time recently being overfilled with wash buffer for computerized washing. Channel- Paper or other retentive fabric were utilized to blotch the plate.

6- Each well take 50 microliters of substrate arrangement A, taken after by 50 microliters of substrate arrangement B. For 10 minutes at 37°C within the dim, the plate was brooded and secured with a new sealer.

7- Each well received 50 microliters of Stop Solution, which caused the blue hue to become yellow almost instantly.

8- Inside 10 minutes after applying the halt arrangement, the optical thickness (OD esteem) of each well was measured employing a microplate peruser set to 450 nm.

3.3.4. IL-9 Levels

Serum levels of IL-9 were estimated by using Bioassay/China ELISA kit in asthmatic patients with mite allergy. Table 3-4 lists the chemical reagents of the kit.

Table 3.4: lists the chemical reagents of the IL-9 kit.

Components	Quantity
Standard Solution (6400pg/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IL-9 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

- Procedure

1- The reagents which incorporate standard arrangements, and tests were arranged concurring to the informational parts. Some time recently utilize, the total reagents were brought to room temperature. The try was carried out at room -temperature.

2- For the test, the number of strips vital was set up. For utilization, the strips were put into the outlines. The unused strips ought to be kept at a temperature of 2-8°C.3- Standard was added to the standard well in the amount of 50 microliters. (Note: Because the standard solution contains biotinylated antibody, the antibody did not contribute to the standard well.)

4- 40 microliter of sample was included to test wells, taken after by 10 microliter of anti-interleukin 9 counter acting agent, 50 microliters of streptavidin-HRP, and at last 50 l of streptavidin-HRP to standard wells (Not clear control well). The plate was well blended, at that point fixed with a sealer and hatched at 37°C for 60 minutes.

5- After expelling the sealer, the plate was washed five times with wash buffer. For each wash, wells were splashed for 30 seconds to 1 diminutive with at slightest 0.35 ml wash

buffer. All wells were suctioned and washed five times with wash buffer some time recently being overfilled with wash buffer for computerized washing. Channel- Paper or other retentive fabric were utilized to blotch the plate.

6- Each well take 50 microliters of substrate arrangement A, taken after by 50 microliters of substrate arrangement B. For 10 minutes at 37°C within the dim, the plate was brooded and secured with a new sealer.

7- Each well received 50 microliters of Stop Solution, which caused the blue hue to become yellow almost instantly.

8- Inside 10 minutes after applying the halt arrangement, the optical thickness (OD esteem) of each well was measured employing a microplate peruser set to 450 nm.

3.3.5. TNF-α Levels

Serum levels of TNF-α were estimated by using Bioassay/China ELISA kit in asthmatic patients with mite allergy. Table 3-5 lists the chemical reagents of the kit.

Table 3.5: lists the chemical reagents of the TNF-α kit.

Components	Quantity
Standard Solution (6400pg/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IL-9 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

- Procedure

1- The reagents which incorporate standard arrangements, and tests were arranged concurring to the informational parts. Some time recently utilize, the total reagents were brought to room temperature. The try was carried out at room -temperature.

2- For the test, the number of strips vital was set up. For utilization, the strips were put into the outlines. The unused strips ought to be kept at a temperature of 2-8°C.3- Standard was added to the standard well in the amount of 50 microliters. (Note: Because the standard solution contains biotinylated antibody, the antibody did not contribute to the standard well.)

4- 40 microliter of sample was included to test wells, taken after by 10 microliter of anti-interleukin TNF counter acting agent, 50 microliters of streptavidin-HRP, and at last 50 l of streptavidin-HRP to standard wells (Not clear control well). The plate was well blended, at that point fixed with a sealer and hatched at 37°C for 60 minutes.

5- After expelling the sealer, the plate was washed five times with wash buffer. For each wash, wells were splashed for 30 seconds to 1 diminutive with at slightest 0.35 ml wash buffer. All wells were suctioned and washed five times with wash buffer some time recently being overfilled with wash buffer for computerized washing. Channel- Paper or other retentive fabric were utilized to blotch the plate.

6- Each well take 50 microliters of substrate arrangement A, taken after by 50 microliters of substrate arrangement B. For 10 minutes at 37°C within the dim, the plate was brooded and secured with a new sealer.

7- Each well received 50 microliters of Stop Solution, which caused the blue hue to become yellow almost instantly.

8- Inside 10 minutes after applying the halt arrangement, the optical thickness (OD esteem) of each well was measured employing a microplate peruser set to 450 nm.

3.4. Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. T-test and Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study.

4. RESULTS AND DISCUSSION

4.1. General Features

The experimental group consists of 100 patients and the control group consists of 50 healthy participants. Of the 100 patients in the experimental group, 62 were male and 38 were female. Of the 50 healthy participants in the control group, 20 were male and 30 were female. The mean of age of the patients group was found to be 35.09 ± 14.59. The mean of age of the control group was found to be 34.18 ± 11.78.

Table 4.1: Result of factors in control and patients groups

Factors		Control	Patients	*P*-value
Gender: No (%)	Male	23 (46.00%)	51 (51.00%)	0.328 NS
	Female	27 (54.00%)	49 (49.00%)	
Age (year)	Mean ± SE	27.86 ± 1.12	32.77 ± 1.35	0.0193 *
* (P≤0.05), NS: Non-Significant.				

4.2. Total IgE Results

There was a significant difference ($P < 0.01$) between asthmatic patients and controls, the mean of total serum IgE levels was 358.82 ± 32.84 in asthmatic patients compare to 79.68 ± 12.31. These results agreed with

Table 4.2: Comparison between control and patients groups in IgE.

Group	No	Mean ± SE of Total IgE
Control	50	79.68 ± 12.31
Patients	100	358.82 ± 32.84
T-test	---	90.789 **
P-value	---	0.0001
** (*P*≤0.01).		

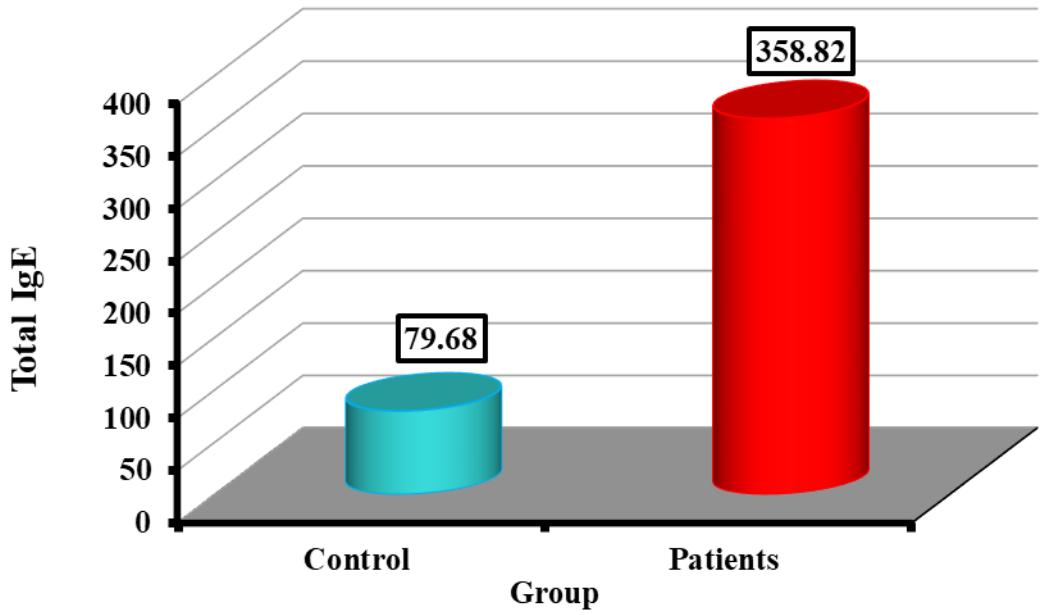

Figure 4.1: Comparison between control and patients groups in Total IgE.

4.3. IL-13, IL-9 and TNF Results

The current results showed that serum level of IL-13 was increased significantly in asthmatic patients (699.53 ± 39.27) group compare to control (11.66 ± 0.73), while serum level of IL-9 was decreased in patients (20.74 ± 1.32) compare to control (491.33 ± 9.04), as illustrated in tables 4.3 and 4.4.

Serum level of TNF was increased significantly in asthmatic patients (94.39 ± 6.05) group compare to control (52.22 ± 1.41) as illustrated in table 4.5.

Table 4.3: Comparison between control and patients groups in IL-13.

Group	No	Mean ± SE of IL-13
Control	50	11.66 ± 0.73
Patients	100	699.53 ± 39.27
T-test	---	109.94 **
P-value	---	0.0001
** ($P \leq 0.01$).		

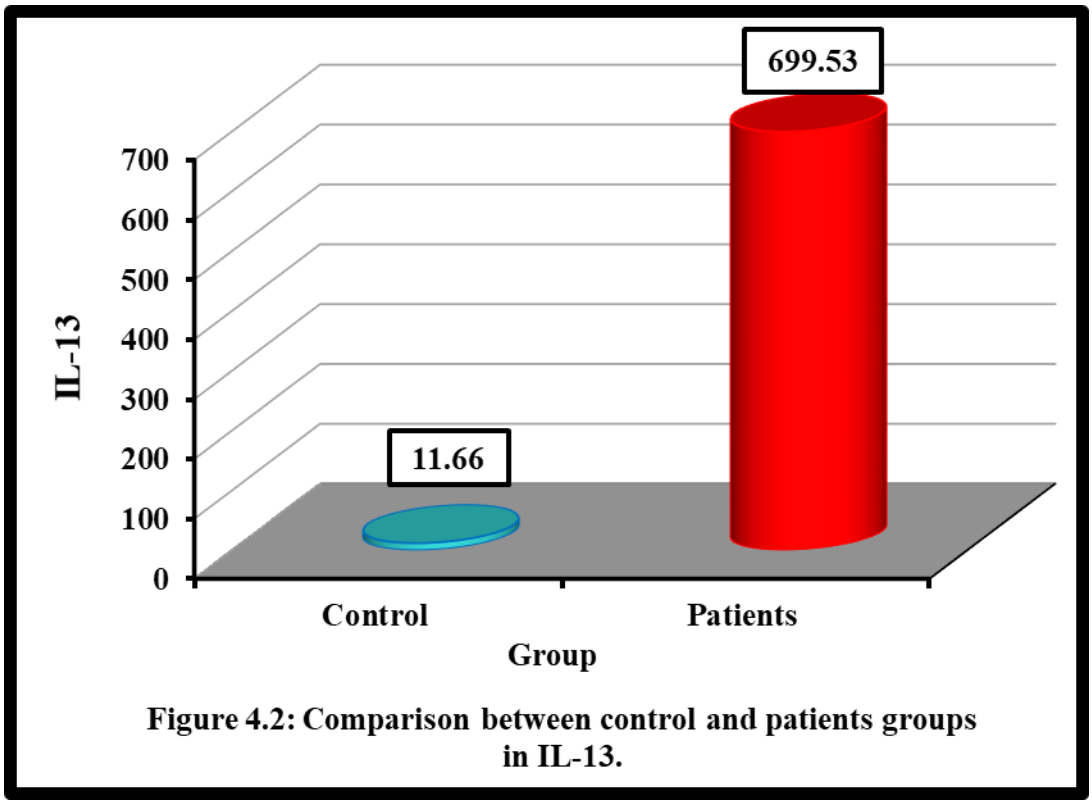

Figure 4.2: Comparison between control and patients groups in IL-13.

Table 4.4: Comparison between control and patients groups in IL-9.

Group	No	Mean ± SE of IL-9
Control	50	491.33 ± 9.04
Patients	100	20.74 ± 1.32
T-test	---	13.134 **
P-value	---	0.0001
** ($P \leq 0.01$).		

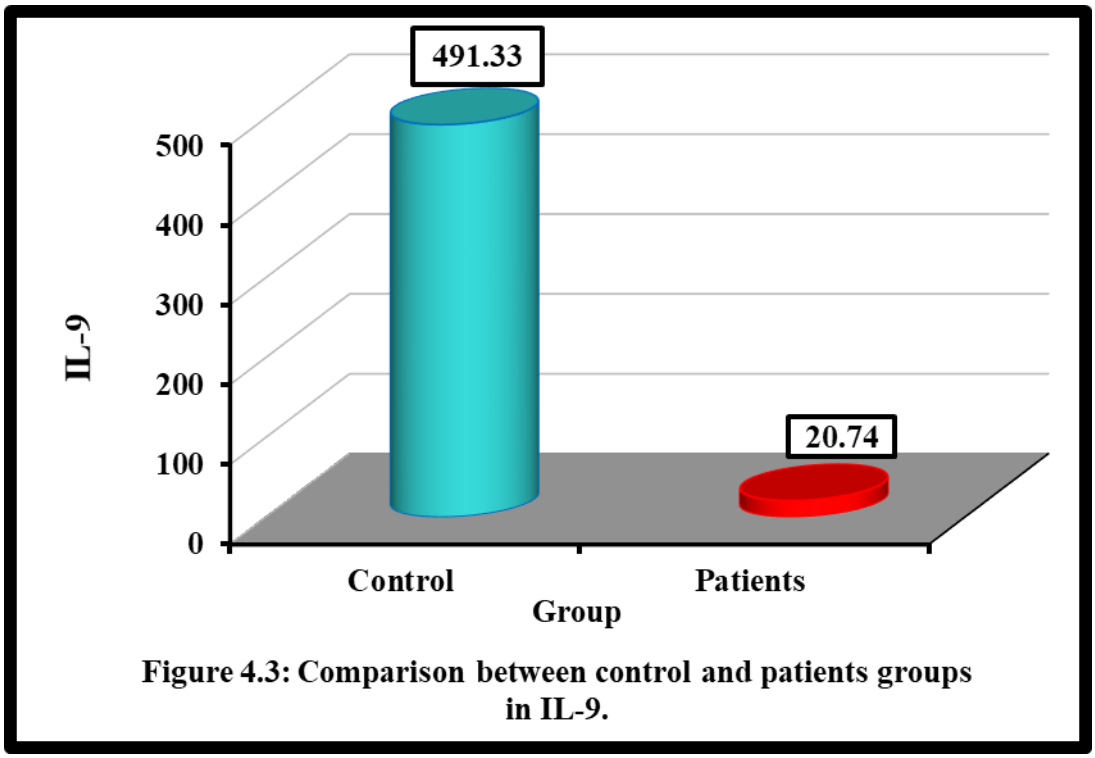

Figure 4.3: Comparison between control and patients groups in IL-9.

Table 4.5: Comparison between control and patients groups in TNF-α.

Group	No	Mean ± SE of TNF-α
Control	50	52.22 ± 1.41
Patients	100	94.39 ± 6.05
T-test	---	17.075 **
P-value	---	0.0001
** ($P \leq 0.01$).		

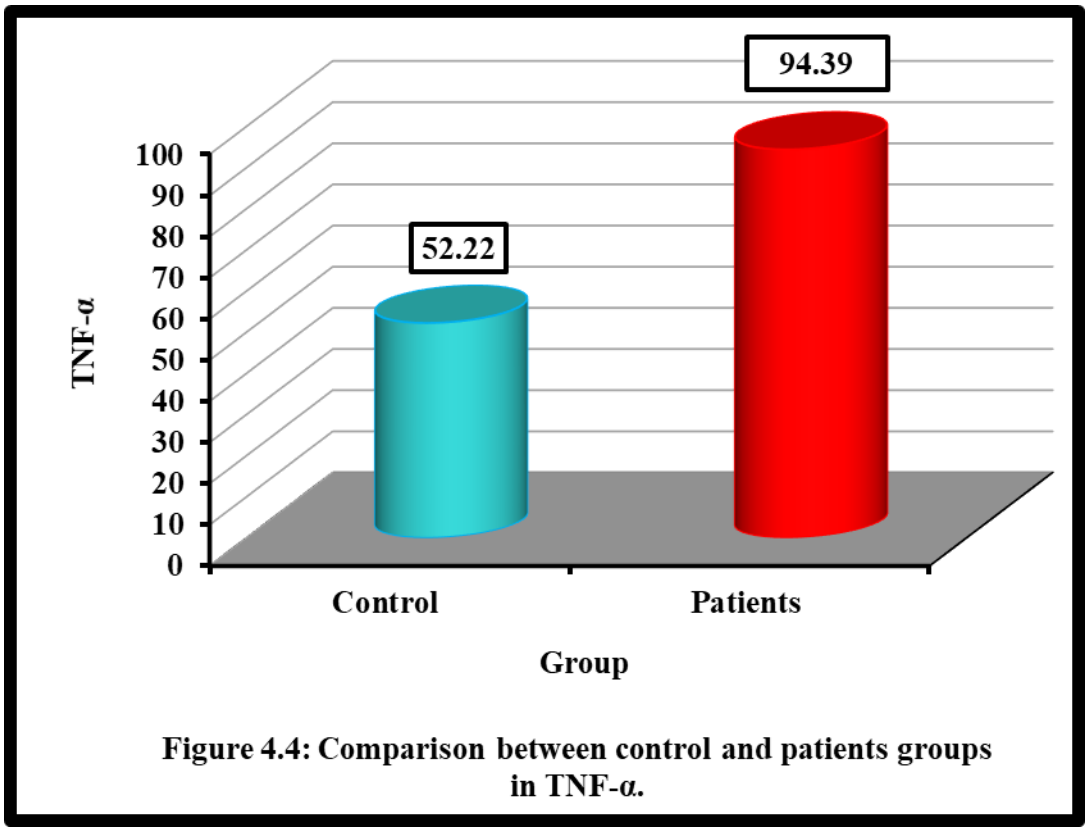

Figure 4.4: Comparison between control and patients groups in TNF-α.

4.4. Estimation of the Studied Parameters According to Gender

According to gender, the serum levels of studied parameters showed non significant difference between male and female, as illustrated in table 4.6.

Table 4.6: Effect of Gender in parameters study in patients group.

Gender	Mean ± SE			
	IgE	IL-9	IL-13	TNF-α
Male	333.93 ±43.17	19.91 ±1.86	704.72 ±42.23	93.01 ±8.90
Female	384.71 ±47.12	21.60 ±1.87	694.13 ±67.49	95.83 ±8.27
T-test	126.84 NS	5.305 NS	157.34 NS	24.075 NS
NS: Non-Significant.				

4.5. Estimation of The Studied Parameters According to Age Groups

According to age groups, the serum levels of studied parameters showed non significant difference among the three age groups, as illustrated in table 4.7.

Table 4.7: Effect of Age groups in parameters study in patients group.

Age groups (year)	Mean ± SE			
	IgE	IL-9	IL-13	TNF-α
Least than 30 yr.	406.88 ±52.44	21.77 ±2.17	725.75 ±69.61	95.35 ±8.86
30-40 yr.	319.53 ±51.82	19.81 ±2.48	626.45 ±35.18	79.35 ±5.12
More than 40 yr.	312.97 ±55.26	19.83 ±2.04	724.28 ±73.54	107.20 ±15.38
LSD value	161.05 NS	6.736 NS	199.78 NS	30.570 NS
NS: Non-Significant.				

4.6. Pearson Correlation Results

The correlation results among the studied parameters showed that, there was strong negative corellation between IgE and IL-9, IL-9 and IL-13, TNF, while strong positive correlation was noticed between IgE and IL-13, IL-13 and TNF, as illustrated in table 4.8.

Table 4.8: Correlation coefficient between difference variables in Patients.

Parameters	Correlation coefficient-r	Sig.
IgE & IL-9	-0.44	**
IgE & IL-13	0.23	**
IgE & TNF-α	0.06	NS
IL-9 & IL-13	-0.69	**
IL-9 & TNF-α	-0.35	**
IL-13 & TNF-α	0.55	**
** ($P \leq 0.01$), NS: Non-Significant.		

Asthma is a chronic inflammatory airway disease associated with the type 2 cytokines interleukin-4 (IL-4), IL-5, and IL-13, which increase airway eosinophilia, mucus overproduction, BHR, and IgE formation. Only half of asthmatics, on the other hand, exhibit signs of a worsening Type 2 response. (Lambrecht et al., 2019). Brakhas, 2015 concluded that allergic patients of any age have significantly higher levels of total IgE than healthy controls, and explained that the immune response to allergens is mediated by IgE antibody specific to the allergen, which activates mast cells and basophils, triggering a series of cellular and molecular events that results in the clinical manifestation of allergic disease. Deo et al., (2010) found that total IgE levels in asthmatic patients were considerably higher than in the general population, and that there is a clear link between IL-4 cytokines and IL-5 in the majority of instances, and that IL-4 is the primary cytokine that causes an increase in IgE production. IL-13 induces goblet cell hyperplasia, increases bronchial epithelial periodic acid schiff (PAS) cell staining, and increases MUC5AC expression in human in vitro studies. Experiments show that these effects are caused by IL-13 signaling via IL-13Rα1. In these studies, IL-13 also produced an increase in the soluble form of IL-13Rα2. IL-13Rα2 inhibited PAS+ cells, MUC5AC+ cells, goblet cells, and both mRNA expression and protein secretion of MUC5AC generated by IL-13Rα2. (Yasuo et al., 2006; Tanabe et al., 2007).

In asthmatic airways, mast cells are a significant source of IL-9. Human eosinophils and neutrophils have also been shown to secrete IL-9. Innate lymphoid cells, which are an essential component of the innate immune system, also produce IL-9. (Gounni et al., 2000; Sun et al., 2018; Turner et al., 2013). TNF-alpha is a proinflammatory cytokine that has been related to a number of asthmatic airway disease features. Evidence is mounting that it may have a key role in the treatment of severe refractory disease. We were able to evaluate the role of TNF-alpha in vivo thanks to the discovery of new TNF-alpha antagonists. Preliminary studies have demonstrated an increase in asthma quality of life, lung function, and airway hyperresponsiveness, as well as a reduction in exacerbation frequency in individuals treated with anti-TNF-alpha therapy. (Brightling et al., 2008). (Th17 cells, asthma, and tumor necrosis factor-a all have a complicated relationship (TNF-α). Patients with severe steroid-resistant asthma have higher levels of TNF-α in their airways, the results of TNF-α blocking in clinical trials have also been variable. (Manni et al., 2014).

In summary, IL-9, IL-13 and TNF are now emerging as potential. Important players in the pathogenesis of lung diseases. In the last two decades, more attention and emphasis has been placed on several other cytokines such as IL-4, IL-5 and IL-17. In contrast, IL-9, IL-13, and TNF have previously been seen as a byproduct of ongoing inflammation in the airways. However, as discussed here, it is clear that IL-9, IL-13 and TNF may play an active role in mediating specific aspects of various lung diseases. In the lung, IL-9, IL-13, and TNF differ from other inflammatory markers. IL-9, IL-13 and TNF may be key modulators of the general immune response as well as the function of non-immune cells.

The most surprising finding was that IgE production was more dependent on endogenously produced IL-9, IL-13, and TNF in the tick allergic asthma group than in the control group. This can be explained by the fact that patients have increased IgE synthesis in response to exogenous IL-9, IL-13, and TNF, as well as increased IL-9, IL-13, and TNF' production. It has a higher susceptibility to IL-9, IL-13, and TNF, as well as more availability. The overwhelming evidence from asthma research supports the idea that the type II cytokines IL-9 and IL-13 play a key role in the pathophysiology of mite allergic asthma.

While IL-9 is important for Th2 cell proliferation, cytokine production, and IgE manufacturing, IL-13 is important for the disease's clinical manifestations. Finally, our findings reveal that exogenous TNF can mimic the adjuvant features of a combustion source particle in a rat lung allergy model, implying that TNF and any other agent that generates a reaction can be used as an adjuvant. In UX, cytokine neutralization only partially reduced eosinophilia and related eosinophil chemotactic cytokines, despite the fact that TNF therapy increased antigen-specific IgE, lymphoproliferative responses, and eventual allergy. TNF-α is required for antigen-specific IgE production and induction of TH2-type cytokines and chemokines in experimental mite allergy. In addition, TNF-a may be important for the expression of adhesion molecules that help recruit eosinophils to the allergic inflammatory site. We conclude that TNF-α is closely involved in mite allergy from its onset to its development.

5. CONCLUSIONS AND RECOMMENDATION

Conclusions

Based on findings of the current study, the following points can be concluded:

1. The current results shows a significant increase of serum IgE levels were clearly observed asthmatic patients.

2. Inhalation of indoor dust mite allergens have affecting role in asthmatic patients.

3. Significant increase in levels of serum IL-13 were observed asthma patients.

4. Significant increase in levels of serum IL-9 were observed asthma patients.

5. Significant increase in levels of serum TNF-α were observed asthma patients.

Recommendations

The current study suggests the following:

1. More studies are required to clarify the possible association between immunological and Molecular factors.
2. Familial study of genotyping for patients with allergic diseases.
3. Focusing on cytokines and anti-cytokines immune therapy trials regarding allergic diseases.

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