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Role of immunological biomarkers IL5, IL17, periostin protein and serum amyloid A1 in phenotyping of pediatric asthma

A thesis

Submitted to the Council of the College of Medicine, University of Kerbala, as Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

By

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بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

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We certify that this M.Sc. thesis titled:

Role of immunological biomarkers IL5, IL17, periostin protein and serum amyloid A1 in phenotyping of pediatric asthma

Was prepared under our supervision in the College of Medicine/

University of Kerbala, as a partial fulfillment of the requirements for the

Degree of Masters of science in Medical Microbiology.

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Chair of microbiology department
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We, the examiners committee, certify that we've read the M.Sc. thesis entitled:

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We have examined the student (**Khalid Jabbar Salim**) in its contents. In our opinion it meets the standards of thesis for the degree of Masters in Medical Microbiology.

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/ /2025

Dedication

I dedicate this work

To the one whom I could never repay for a blessing I received... My Creator and Lord

To the Prophet Muhammad and his Household (peace be upon them).

To the awaited Imam Mahdi (peace be upon him).

To the one whom... God guided me with his prayers……. My dear father.

To the one who took my hand to the good of this world and the hereafter

My late mother.

To everyone who stood by my side on the path of knowledge....

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Summary

Asthma is a persistent bronchial disorder characterized by inflammation of the airway that is completely or partially reversible Airway hyperactivity in a response to a variety of stimuli.

This study aimed to evaluate the role of immunological biomarkers, including interleukin-5, interleukin-17, periostin protein, total immunoglobulin E , specific immunoglobulin E for inhaled allergens, and serum amyloid A1 ,in the phenotyping of pediatric asthma.

A cross-sectional study on a sample of asthmatic children aged 5-15 years, attending the Center of pediatric Pulmonology at Kerbala Teaching Hospital pediatric, Iraq, from October 2023 to July 2024. The study was included 100 asthmatic children, divided into four groups (eosinophilic, neutrophilic, mixed granulocytic, and allergic) based on immunological markers. The patients with systemic corticosteroids, with autoimmune diseases, or upper/lower respiratory infections was excluded.

Tests ELISA technique Conducted on Blood samples analyzed for immune biomarkers (serum amyloid A1, interleukin-17, interleukin -5, periostin protein, and total immunoglobulin E). While the specific IgE using micro array strips with allergens.

The majority of patients were male (75%) and aged 9-12 years (58%). Urban residence was predominant (65%). A family history of asthma was significantly p-value associated (70%). Eosinophilic Group had higher levels of interleukin -5 and periostin, indicating eosinophilic inflammation. Neutrophilic Group had serum amyloid A1 and interleukin-17 were significantly elevated, linked to neutrophilic airway inflammation. Mixed granulocytic phenotypes exhibited distinct inflammatory profiles. Common aeroallergens were included Russian thistle (22%) and cat dander (18%). interleukin-5 levels were significantly elevated in certain aeroallergen-positive groups, e.g., sorrel and white ash.

Elevated priostin levels in serum correlate with eosinophilc airway inflammation. Positive correlations were observed between serum amyloid A1, interleukin-17, interleukin -5 and periostin. Negative correlations were noted between Total immunoglobulin E and other biomarkers

High incidence of asthmatic pediatric patients in range (5-15), were (9-12) old with urban residence. Great association between increase level of interleukin -5 and priostin in eosinophlic asthmatic patients, while serum amyloid A1 and interleukin -17 increase in neutrophilic asthmatic patients.

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Abbreviations	Meaning
AD	Allergic diseases
AHR	Allergic hyperresponsiveness
AIT	Allergy immunotherapy
APCs	Antigen presenting cells
AUC	Area under the curve
BHR	Bronchial hyperresponsiveness
CBC	Complete blood count
CCD	cross-reactive carbohydrate determinant
COPD	Chronic obstructive pulmonary disease
DC	dendritic cell
EBC	Eosinophil blood count
ECs	*Epithelial-derived cytokines*
EDN	Eosinophil-derived neurotoxin
EIA	Enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ERK	Extracellular signal-regulated kinase
FEV1	Forced Vital Capacity
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GM- CSF	Granulocyte-macrophage colony-stimulating factor
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IgG	Immunoglobulin g
IL-17	Interleukines 17
IL-5	Interleukines 5
ILC2	Innate lymphoid cells types-2
ILs	Interleukins
JNK	Janus Kinase
LPS	lipopolysaccharides
MBP	major binding protein

NK	Natural killer
OD	Optical density
PA	Pediatric asthma
PPV	Positive predictive value
ROC	receiver operating characteristics
RSV	Respiratory syncytial virus
RTIs	Respiratory tract infection
RV	Rhinovirus
SAA1	Serum amyloid A1
Std	Stander deviation
TGF-β	Transforming growth factor β
Th	T-helper cells
Th17	T helper 17
T-regs	T- regulatory cells
TSLP	Thymic stromal lymphopoietin
URT	Upper respiratory tract

Chapter one

Literature Review

1.1. Introduction

Asthma is a chronic respiratory condition characterized by airway inflammation, hyper responsiveness, and variable airflow obstruction (Cevhertas *et al.*, 2020). While asthma incidence and prevalence are higher in children, morbidity, and mortality are higher in adults. Childhood asthma is more common in boys while adult asthma is more common in women, and the reversal of this sex difference in prevalence occurs around puberty suggesting sex hormones may play a role in the etiology of asthma (Dharmage, Perret and Custovic, 2019).

Despite significant advancements in understanding its pathophysiology, asthma remains a highly heterogeneous disease, particularly in children (Cevhertas *et al.*, 2020). This heterogeneity underscores the importance of phenotyping, which involves classifying asthma based on underlying pathobiological mechanisms rather than clinical symptoms alone (Gonzalez-Uribe et al., 2023). Accurate phenotyping can aid in personalized treatment strategies and improve disease management outcomes (Ahmed, 2022).

Immunological biomarkers have emerged as valuable tools in phenotyping pediatric asthma. Among these, interleukin-5 (IL-5) and interleukin-17 (IL-17), IL-5 plays a central role in the differentiation, activation, and survival of eosinophils, a specific type of white blood cell involved in allergic inflammation (Gohal *et al.*, 2024). In this context, a subset of T cells mainly produces IL-17, called Th17 cells. In asthma, IL-17 promotes the recruitment and activation of neutrophils, another type of white blood cell. Neutrophils contribute to airway inflammation and tissue damage. (Fan *et al.*, 2023)

Periostin, an extracellular matrix protein induced by IL-13 and IL-4, serves as a marker of airway remodeling and Th2-driven inflammation (Li *et al.*, 2015). Similarly, total immunoglobulin E (T.IgE) and specific IgE to inhaled allergens provide insights into atopy, a major driver of allergic asthma phenotypes (Rabin and Levinson, 2008; Hoshino *et al.*, 2022). Serum amyloid A1 (SAA1), an acute-

phase reactant, has been associated with neutrophilic inflammation, highlighting its relevance in non-allergic asthma subtypes (Draijer *et al*., 2013).

The integration of these biomarkers facilitates the identification of asthma phenotypes, such as eosinophilic, neutrophilic and allergic asthma in children (Carr, Zeki and Kraft, 2018). This phenotypic classification enables a more precise understanding of the disease, guiding targeted therapeutic interventions such as monoclonal antibodies directed at IL-5, IL-4, and IgE pathways (Roufosse, 2018).

Children with asthma may impair airway development and reduce maximally attained lung function, and these lung function deficits may persist into adulthood without additional progressive loss (Dharmage, Perret and Custovic, 2019).

1.2.1. Aim of study

The aim of this study was planned to evaluate the role of immunological biomarkers, including interleukin-5 (IL-5), interleukin-17 (IL-17), periostin protein, total immunoglobulin E (T.IgE), specific IgE for inhaled allergens, and serum amyloid A1 (SAA1), in the phenotyping of pediatric asthma.

These study objectives achieved by:

1-ELISA technique for SAA1, IL-17, IL-5, priostin and Total IgE, while

2-Specific Aeroallergens inhalation detection is done by micro array of assay strips (EUOROIMMUNE).

3-Complete blood count (CBC) five differential by Sysmex XN-350 automated haematology analyzer (Sysmex, Japan) to count white blood cells.

4-Pulmonary function test by: Spirometery for lung function test.

5-Fractional Exhaled Nitric Oxide test (FeNO) by a novel medical technology.

1.2. Definition of asthma

Asthma is a consequence of complex gene–environment interactions, with heterogeneity in clinical presentation and the type and intensity of airway inflammation and remodeling. (Papi *et al.*, 2018). Asthma is a heterogeneous disease characterized by bronchial hyper-reactivity, chronic airway inflammation, and reversible airflow obstruction, and it affects individuals in all age groups. (Ricciardolo *et al.*, 2023) , which characterized by cough, wheeze, chest tightening, and shortness of breath. It is also characterized by some other symptoms like epithelial rupture, hypertrophy of airway smooth muscles, hyper secretion of mucous in lungs bronchial walls (Foppiano and Schaub, 2023) . That leads to significant morbidity, mortality, and financial burden.

1.2.2. History of asthma

The word “asthma” originates from the Greek meaning short of breath, meaning that any patient with breathlessness was asthmatic.(Holgate, 2010) . In the 1900s, selective beta-2 adrenogen receptors agonists were used for asthma treatment (Kapri *et al.*, 2023). In 1920s, the deaths from asthma were related to airway remolding and extensive inflammation (Kapri *et al.*, 2023) . In 1960s–1970s, technological advancements led to the use of peak flow meters to measure obstruction in airways and arterial blood gasses.(Kapri *et al.*, 2023).

1.2.3. Epidemiology of asthma

Asthma affects more than a quarter of a billion people worldwide, is the most common chronic condition in childhood, and is responsible for over 1000 deaths a day, of which the majority are preventable(Levy *et al.*, 2023). This condition leads to significant morbidity, mortality, and economic strain worldwide. (Gonzalez-Uribe, Romero-Tapia and Castro-Rodriguez, 2023). The number of people suffering from asthma is more than the number of people suffering from HIV infection and tuberculosis.(Kapri *et al.*, 2023) .

In Iraq, The International Study of Asthma and Allergies in Childhood (ISAAC) recorded the prevalence of clinically diagnosed childhood asthma as 16.3% in primary school children (Abood and Al-Zaubai, 2020) , (figure 1). the prevalence of asthma in Iraq is rather high compared to nearby and neighboring countries.in Islamic Republic of Iran the childhood asthma was ranging 6% to 8%, and the prevalence in boys and girls was 9% and 8%, respectively(Rahimian *et al.*, 2021) . While, in Jordan The prevalence of asthma was 2.38% amongst both sex, (Nour, Alsayed and Basheti, 2023) and 15% in Kuwait (Alavinezhad and Boskabady, 2018) , in Syria it was 19.28 %.(Mohammad *et al.*, 2023) . The prevalence of children asthma in Saudi Arabia varies among different regions throughout the country. The highest prevalence was reported in Alhofuf (33.7%) and the lowest in Abha (9%).(Alahmadi, Banjari and Alharbi, 2019). the prevalence of pediatric asthma in Turkey was estimated to be 7.4 %.(Şekerel *et al.*, 2020)

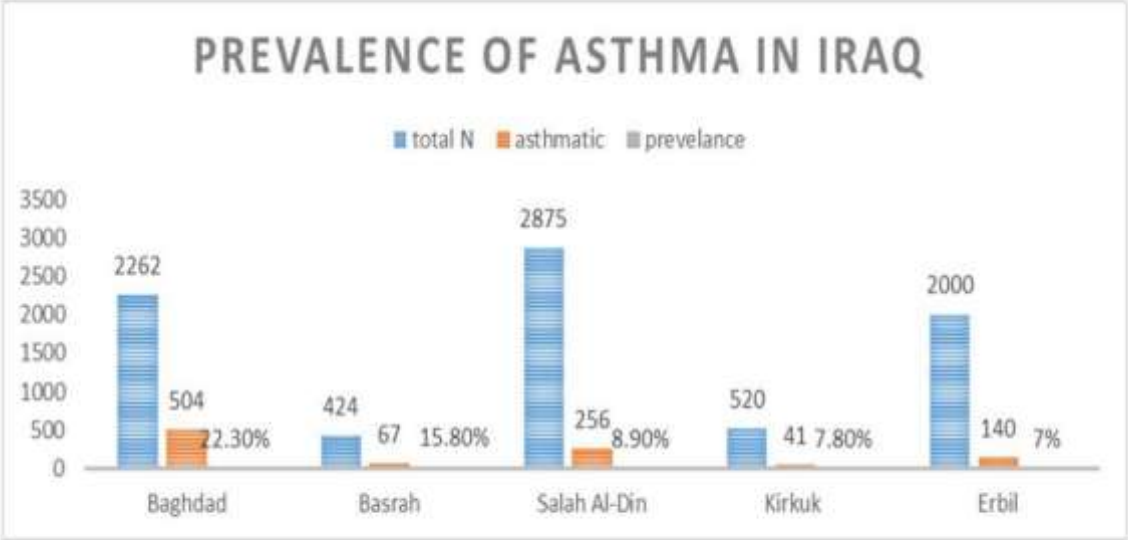


FIGURE 1. - The prevalence of asthma in Iraq

Figure 1.1:The prevalence of asthma in Iraq (Alsajri, Al-Qerem and Mohamed Noor, 2023)

Not far from this regions, the Asian countries also had levels of burdens from asthma, the prevalence of childhood asthma in china 1.11% in girls and 10.27% in boys (Li *et al.*, 2020) . While In India the estimated prevalence of asthma was

7.9%(Alavinezhad and Boskabady, 2018) .In Australia, the prevalence of asthma among children was 11% (Figure 2).

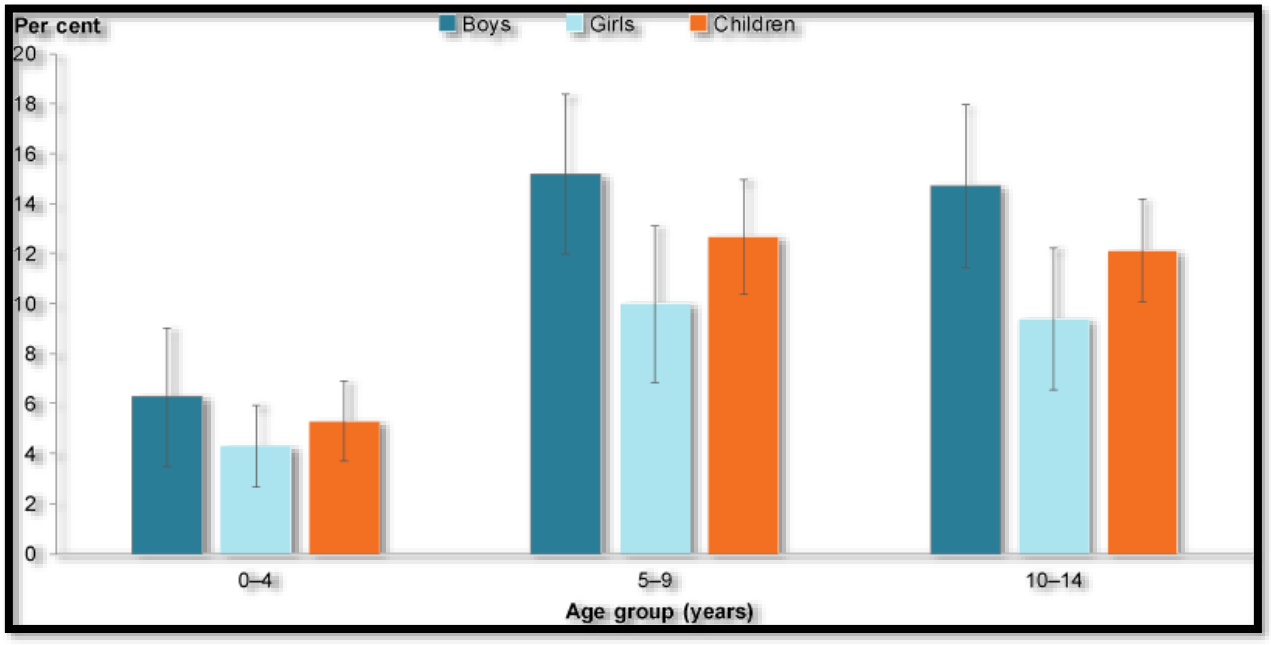


Figure 1.2: Asthma prevalence is based on having been diagnosed by a doctor or nurse as having asthma (current and long term). Source: AIHW analysis of ABS 2018.

The prevalence of asthma among children in Korea 8.65%.(Kim *et al.*, 2023), the prevalence of childhood asthma in southern Africa is variable but has increased over the last four decades, particularly in South Africa (3.17% to 21.29%) while the Prevalence of asthma among Egyptian children of 7.7% (8% in urban and 7% in rural areas). In USA, children's asthma (6.5%) (Xie *et al.*, 2020). Overall Europe, the prevalence of asthma ranges from 5.1% to 8.2% in adults, although large differences exist between European countries, with 1.3% in Bosnia and 17.6% in the United Kingdom (UK) (Wecker *et al.*, 2023) .

1.2.4. Etiology of asthma

Clinical features of asthma and its immunological and molecular etiology vary significantly among patients. (Barcik *et al.*, 2020).Bacteria and viruses are the most microorganisms extensively studied relating to asthma pathogenesis, but other microbes, including fungi and even Archaea, can potently influence airway inflammation.

There is a large body of evidence demonstrating a link between early viral infections (especially Rhinovirus (RV) and respiratory syncytial virus (RSV) and asthma inception and exacerbations. (Mikhail and Grayson, 2019). Rhinovirus infections are potent triggers for acute airway obstruction and exacerbations in children and adults. (Jackson and Gern, 2022). while (RSV) is the leading cause of lower respiratory tract infection in children and is a common cause of wheezing in infants and young children.(Knudson and Varga, 2015) . Although viruses are the most common asthma exacerbation triggers, Mycoplasma pneumonia, which have been proposed as possible risk factors or contributors, there was a statistically significant association between M. pneumonia infection, with increased risk of any type of childhood asthma. (Liu *et al.*, 2021) .in the other hand, there is definite evidence of fungal sensitization in asthma, Aspergillus species seem to be the strongest candidates.(Agarwal and Gupta, 2011) . The clinical outcomes of allergy to aeroallergens such as Alternaria and Cladosporium are predictable from their spore levels and cause short-term allergic manifestations similar to those induced by grass pollen. (Rick *et al.*, 2016).

1.2.5. Risk factors

Many risk factors have been associated with asthma and the differences in distributions of these risk factors may explain the differences in prevalence.(Stern, Pier and Litonjua, 2020).

1.2.5. A. Genetics

Asthma runs in families, and children of asthmatic parents are at increased risk of asthma. (Thomsen, 2015). Recent large-scale genome-wide association studies have successfully identified several genetic loci that influence asthma susceptibility.(Cookson, Moffatt and Strachan, 2011).

1.2.5. B. Vitamin D

Vitamin D is a potent immunomodulator capable of dampening inflammatory signals in several cell types involved in the asthmatic response.(Hall, Agrawal and Author, 2017) .

Over the last decade, there has been increasing interest in the role of vitamin D in asthma. Clinically, the association between vitamin D and fetal lung development has been also revealed in both animal and fetal models[4] (Esfandiar *et al.*, 2016) .Vitamin D deficiency is associated with increased inflammation, exacerbations, and overall worse outcomes in pediatric asthma and is observed in asthmatic children (Sung, 2023). It dampen the effects of both the innate and adaptive immune responses through its effects on dendritic cells, macrophages, and T and B lymphocytes. also play a role in asthma pathogenesis by affecting the development of the lung, regulating the immune responses, and remodeling of airway smooth muscle .(Salmanpour *et al.*, 2022) .Vitamin D deficiency can affect Th1 and Th2 cytokines, which may also contribute to the development of atopy (Bantz, Zhu and Zheng, 2015). Studies also suggest an inhibitory effect of vitamin D on Th17 response. (Bantz, Zhu and Zheng, 2015).

1.2.5. C. Air pollution

Air pollution is the result of a complex mixture of pollutants that includes solid and liquid particles suspended in the air. (Tiotiu *et al.*, 2020) . Various pollutants have been incriminated including ozone, nitrogen dioxide (NO2), particulate matter (PM) and others.(Dharmage, Perret and Custovic, 2019). Epidemiologic studies of air pollution and asthma have identified increased risk of both exacerbation of lung disease with acute exposure. The mechanisms have been proposed induced by the interaction of pollutants with the epithelium of the bronchial tree and the generation of oxidative stress, and/or by immunological mechanisms mediated by interaction with dendritic cells or type 2 innate lymphoid cells (ILC-2) and T-helper 2 (Th2) or 17 (Th17) lymphocytes.[17] (Tiotiu *et al.*, 2020).Importantly, pollutants (e.g., O3, SO2) can act as adjuvants and affect the production of some cytokines in airway epithelial cells, which promote T-helper 2 (Th2) phenotypic differentiation and IgE production. (Tiotiu *et al.*, 2020) . Pollen is one of the main reasons to cause seasonal allergic asthma and influenced by multiple risk factors. (Xie, Guan and Yin, 2019).

1.2.5. D. Smoking

Exposure to tobacco smoke stimulates the immune response that can co-occur with asthma, lead to the development of asthma-like symptoms.(Nagase, Yamashita and Ohta, 2012) . Which induces oxidative stress and the release of pro-inflammatory mediators by activated neutrophils, macrophages, and CD8+ cytotoxic T cells. (Thomson, Polosa and Sin, 2022), Secondhand smoke (SHS) exposure can trigger asthma exacerbations in children, there is a positive association between prenatal and postnatal secondhand smoking exposure and the occurrence of childhood asthma , Passive smoking significantly reduced the levels of FoxP3 (Forkhead/winged helix transcription factor) and tumor growth factor-β, which were associated with T-reg cells, and increased the levels of interleukin-17A and interleukin-23, which were associated with Th17 cells. The levels of Environmental tobacco smoke (ETS) and serum IgE, and the duration and amounts of passive smoking were closely associated with asthma severity..

1.2.5. E. Drugs

Current evidence on the effect of systemic and local β-blockers on asthma outcomes. (Tiotiu *et al.*, 2019). The potential for asthma exacerbation is one of the major adverse effects of beta-blockers. (Huang *et al.*, 2021). Non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (NERD) is characterized by moderate-to-severe asthma ,and Aspirin challenge is the gold standard to diagnose NERD.(Woo, Luu and Park, 2020)

1.2.5. F. Obesity

Obesity, defined as a body mass index (BMI) ≥ 30 kg/m (Tooba and Wu, 2022). Which is a vast public health problem, and both a major risk factor and a disease modifier for asthma in children and adults. (Sansone *et al.*, 2020) .Sex does not appear to modify this risk. (Ahmadizar *et al.*, 2016) . Obesity is associated with airway dysanapsis, in which airway caliber is smaller and disproportionate to lung volume, which is in turn associated with higher risk of asthma exacerbation. (Tooba and Wu, 2022) .

Obesity-related asthma is considered to be non-Th2-related, because obesity induces a Th1 polarization among CD4 cells, leading to a predominant Non Eosinophilic Asthma (NEA) with no atopic or Th2-related previous responses. (Antolín-amérigo and Quirce, 2018)**.**

1.2.6. Classification of Asthma

Several classifications of asthma have been proposed over the years, based on the Etio-pathogenesis (allergic or non-allergic), inflammatory pattern (eosinophilic or non-eosinophilic), and more recently, in the underlying distinctive pathophysiologic mechanism (type 2-high or type 2-low). (Bush, 2019) . Allergic asthma is triggered by an allergic reaction to allergens such as pollen or pet dander. (Novosad and Krčmová, 2020) . Nevertheless, between 10 and 33% of asthmatic individuals have nonallergic asthma (Baos *et al.*, 2018) . it is triggered by factors other than allergens, like cold and dry air, respiratory infections, hormonal changes, smoke and air pollution. (Klain *et al.*, 2022).

Asthma can be classified into either intermittent or persistent, and the latter is either mild, moderate, or severe. Severe asthma is defined as "asthma which requires treatment with high dose inhaled corticosteroids (ICS) plus a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled,' or which remains 'uncontrolled' despite this therapy." (Yilmaz, Çetin and Arslan, 2022). While, Mild asthma defined as Well-controlled with as-needed reliever medication alone or with low-intensity controller treatment such as low-dose inhaled corticosteroids (ICSs), leukotriene receptor antagonists, or chromones . Based on the cellular activity, asthma can be classified into other branch; Eosinophlic asthma, Non eosinophlic asthma or Neutrophilic asthma.

1.2.6. A. Eosinophilic asthma

Most asthmatics have type 2 inflammation, which is associated with certain cytokine profiles (IL-4, IL-5, and IL-14) and inflammatory cells (eosinophils, mast cells, basophils, type 2 T helper lymphocytes, and immunoglobulin E

[IgE]-producing plasma cells). (Mims, 2015)**.** An individual may be predisposed to the effect of an irritant or allergen. Major histocompatibility complex (MHC) class II antigens may allow specific inhaled allergens to be presented more effectively to T lymphocytes by monocytes or dendritic cells.(Bush, 2019) .

After being primed by the T cell and antigen-presenting cell, the B cell is directed to produce allergen-specific IgE. This IgE is released into the blood and quickly binds to high-affinity IgE receptors (FcεRI) on the surface of mast cells and peripheral blood basophils and to the low-affinity IgE receptors (FCεRII or CD23) on the surface of lymphocytes, eosinophils, platelets, and macrophages(Hammad and Lambrecht, 2021). Once mast cells are coated with antigen-specific IgE, future exposure to the antigen will lead to mast cell activation. When allergen-specific IgE molecules on the surface of the mast cells interact with allergen, a cross linking of the IgE occurs. This cross linking induces the activation of signaling cascades and causes the release of preformed granules containing histamine, tryptase, chymase, eicosanoids, free radicals, and preformed Th-2–like cytokines(Ando and Kitaura, 2021).

This exocytosis of preformed mediators constitutes the “early phase reaction.” These substances are quite toxic and are responsible for acute asthmatic symptoms. Histamine induces the contraction of airway smooth muscle, mucus secretion, and vasodilatation. A loss of microvascular integrity follows and plasma proteins and plasma leak into the airway walls, causing lumen narrowing Chymase has a procollagen proteinase activity and is probably directly toxic to the airway cells (Ray and Kolls, 2017). The release of the cysteinyl leukotrienes as well as other inflammatory cytokines leads to the “late phase reaction,” which primarily involves the recruitment and activation of eosinophils, Th-2–type CD4+ cells, macrophages, and neutrophils (Asamoah *et al.*, 2024).

Once the inflammatory reaction or late phase is initiated, eosinophils become one of the major mediators of chronic inflammation in allergic asthma. higher doses of antigen, which are often seen in aerosol and food exposures, tend to increase IL-4 and produce a Th-2–type reaction (Walford and Doherty, 2014) ,IL-4–producing Th-2 cells induce Th-2 phenotype amplification and inhibit the Th-1 response. (Bush, 2019) . IL-5 which is produce in large amounts by group-2 innate lymphoid cells (ILC2s). (Nakagome and Nagata, 2024), is the cytokine primarily responsible for selective differentiation of the eosinophil. (Mims, 2015).

Four substances attract eosinophils into inflamed tissue: leukotriene B4, IL-16, and eotaxin 1 and 2. Once in the airways, eosinophils can release a cornucopia of toxic granules that cause direct tissue damage, smooth muscle contraction, and increased vascular permeability, ultimately leading to the recruitment of more eosinophils and Th-2–type cells to the airway. Airway changes include wall thickening, subepithelial fibrosis, increased mucus production and goblet cell mass, myofibroblast hyperplasia, myocyte hyperplasia and hypertrophy, and epithelial cell hypertrophy. Functionally, a thickened airway wall may cause more narrowing than a thin wall. IL-4 is involved in T-cell differentiation, B-cell activation, B-cell differentiation into plasma cells, and the production of immunoglobulin E .(Antolín-amérigo and Quirce, 2018). In the lungs, type-2 cytokines, including IL-4, IL-5, and IL-13, are mainly produced by T helper (Th) 2 cells and T follicular helper cells. (Hammad and Lambrecht, 2021).

1.2.6. B. Neutrophilic asthma

Neutrophilic asthma is a phenotype of asthma that is very severe and persistent, with frequent exacerbations, and characterized by fixed airway obstruction. (Syabbalo, 2020). It is characterized by the presence of high levels of neutrophils in the lungs and airways and fixed airflow obstruction. Activated neutrophils release multiple proteinases, cytokines, chemokines and reactive oxygen species which cause airway epithelial cell injury, inflammation, hyperresponsiveness and airway remodeling.

Neutrophilic asthma or non-eosinophilic asthma has been associated with environmental and/or host factors, such as smoking cigarettes, pollution, work-related agents, infections, and obesity. (Antolín-amérigo and Quirce, 2018) . Airway neutrophilia has been associated with persistent airflow obstruction. Neutrophil recruitment and activation into the airways have been related to the stimulation of toll-like receptor signaling and activation of innate immunity, causing a shift toward Th1 and Th17 responses. This process leads to increased production of interleukin (IL)-8, IL-17A, neutrophil elastase, and a form of matrix metalloproteinase-9 (MMP-9) that shows reduced inhibition by tissue inhibitors of metalloproteinases. These cytokines and activated enzymes, acting together, can modify airway structures to contribute to the lower FEV1, remodeling, and fixed airway obstruction seen in adult patients with severe neutrophilic asthma. (Antolín-amérigo and Quirce, 2018)**.**

1.2.7. The immune system: innate and adaptive immunity

The immune system refers to a collection of cells, chemicals and processes that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms) such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins, the immune system can be simplistically viewed as having two "lines of defense": innate immunity and adaptive immunity.(Marshall *et al.*, 2018)

Innate immunity represents the first line of defense to an intruding pathogen. It is an antigen-independent (nonspecific) defense mechanism that is used by the host immediately or within hours of encountering an antigen. The innate immune response has no immunologic memory and, therefore, it is unable to recognize or "memorize" the same pathogen should the body be exposed to it in the future.(Arjomandnejad *et al.*, 2023).

Adaptive immunity, on the other hand, is antigen-dependent and antigen-specific and, therefore, involves a lag time between exposure to the antigen and maximal response. The hallmark of adaptive immunity is the capacity for memory

which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen. Innate and adaptive immunity are not mutually exclusive mechanisms of host defense. (Marshall *et al.*, 2018)

The cells of the adaptive immune system include: antigen-specific T cells, which are activated to proliferate through the action of APCs, and B cells which differentiate into plasma cells to produce antibodies. Several types of T cell responses can be induced by an APC, with Th1, Th2 and Th17 being the most frequent. The Th1 response is characterized by the production of IFN-γ which activates the bactericidal activities of macrophages and enhances anti-viral immunity as well as immunity to other intracellular pathogens.

Th1- derived cytokines also contribute to the differentiation of B cells to make opsonizing antibodies that enhance the efficiency of phagocytes. (Fahy, 2015). The Th2 response is characterized by the release of cytokines (IL-4, 5 and 13) which are involved in the development of immunoglobulin E (IgE) antibody producing B cells, as well as the development and recruitment of mast cells and eosinophils that are essential for effective responses against many parasites. In addition, mast cells and eosinophils are instrumental in the initiation of acute inflammatory responses, such as those seen in allergy and asthma. IgE antibodies are also associated with allergic reactions.(Sharonov *et al.*, 2020)

1.2.8. Pathophysiology

The pathogenesis of asthma is complex and not fully understood. It involves several immune cells including the T-lymphocytes, B-lymphocytes, mast cells, eosinophils, neutrophils, macrophages, dendritic cells (DCs), etc.(Fireman, 2003)

Inflammation of the bronchial wall involving eosinophils, mast cells, and lymphocytes, as well as their cytokines and inflammatory products, provokes hyper-responsiveness of the bronchi, exacerbating them to narrow more readily in response to a variety of stimuli.(Mahajan and Mehta, 2006).

Dendritic cells (DCs) are the immune cells, and they are known for their role to induce the primary immune responses. Immature DCs are present throughout the lungs and are vital in the immune response to inhaled antigens. DCs are involved in the differentiation of the Th cells into Th2 cells and are the key mediators of airway inflammation (figure 1-3). (Humeniuk, Dubiela and Hoffmann-Sommergruber, 2017).

When the naïve T cells come across the antigen, they differentiate into the effector T cells and memory T (Tm) cells. The effector cells consist of several cells including the Th1, Th2, Th17, Th22, Th9, Th25, T regulatory (Treg), T follicular helper, natural killer T cells, and cytotoxic CD8+ T lymphocytes. These effector cells regulate the innate immune cells (macrophages, eosinophils, mast cells, basophils) and stimulate the B cells. The T cells are also responsible for the generation of cytokines and chemokines to intensify the immune response leading to the escalation of smooth muscle contraction, mucus airway hyperresponsiveness, and airway obstruction (Zhu *et al.*, 2020).

The imbalance between the Th1/Th2 cells plays a significant role in asthma pathogenesis. Interleukin 12 and interferon (IFN)-γ stimulate the T-bet to activate the Th1 cells, whereas IL-4 to activate Th2 cells. Th1 cells produce IL-2, IFN-γ, and lymphotoxin (LT-) α, that further promote type 1 immunity.

They play two regulatory roles in asthma: they suppress Th2 cell activation to limit eosinophilic inflammation while promoting neutrophilic inflammation. Airway epithelial derived cytokines (IL-33, IL-25, and thymic stromal lymphopoietin) stimulate Th2 cells. Subsequently secreting Th2-associated cytokines (IL-4, IL-5, and IL-13). IL-4 and IL-13 urge B cells to produce immunoglobulin E (IgE), mucus secretion, and Airway hyper-responsiveness (AHR).(Bakakos *et al.*, 2023).

Mast cells are found throughout the body in mucosal and epithelial tissues, also can be found in the peritoneal and thoracic cavities of rodents. When which become activated, mast cells are responsible for the secretion of the various

mediators including histamines, proteases, proteoglycans, leukotriene B4 , leukotriene, prostaglandin D2, platelet-activating factor, cytokines (including IL-4, IL-5, and IL 13), and superoxide dismutase which is capable of inducing bronchoconstriction, mucus secretion, and mucosal edema, regulating both IgE synthesis and the development of eosinophilic inflammation. When it comes to bronchial asthma, mast cells play a significant role in both early-phase reaction and late-phase reaction of asthma. (Redegeld *et al.*, 2018)

Eosinophils play a significant role in the pathophysiology of asthma by the secretion of lipid mediators, proteins, and other factors. Eosinophils are the storehouse of the four proteins in their granules, eosinophils store four basic proteins: major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO). MBP is toxic to the respiratory epithelial cells and pneumocytes. It has been observed that mild asthma is characterized by elevated numbers of eosinophils, both in the bronchial mucosa and in bronchoalveolar lavage fluid (BALF). (Shinde, Wankhede and Vyawahare, 2023).

The four inflammatory phenotypes of asthma can be classified based on this quantification: eosinophilic (high eosinophils, normal neutrophils), neutrophilic (high neutrophils, normal eosinophils), mixed granulocytic (high eosinophils, paucigranulocytic high (normal neutrophils), eosinophils, and normal neutrophils).(Fricker and Gibson, 2017).

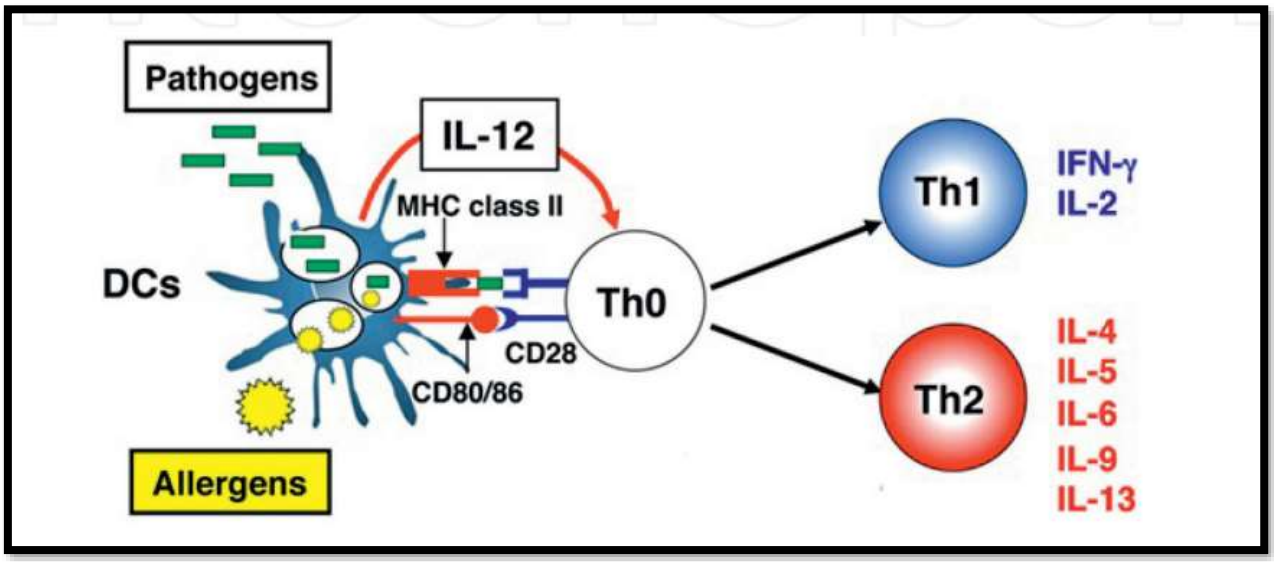


Figure 1.3:. Dendritic cells as antigen-presenting cells (Yoshimoto, 2018)

1.2.9. Immunological markers

The identification of biomarkers can potentially aid diagnosis and inform prognosis, help guide treatment decisions, and allow clinicians to predict and monitor response to treatment.(Oppenheimer *et al.*, 2022).

1.2.9.1. Immunoglobulin E

IgE is the hallmark of type 1 hypersensitivity, IgE synthesis is thought to occur through different biosynthetic pathways, either by "direct" class-switch recombination from IgM, in germinal center B-cells, or through "sequential" switch from IgM to IgG1 and then from IgG1 to IgE, which may occur outside of germinal centres(Froidure *et al.*, 2016) . Allergen-specific IgE binds to its cognate receptors, thus triggering a series of cellular events, which include presentation of antigen by dendritic cells and the degranulation of mast cells and basophils to release numerous mediators that related to disease symptoms(Owen, 2007). IgE secretion by B lymphocytes defines the allergic state and nearly all asthmatics have higher than normal IgE levels in serum. (Owen, 2007) .Exposure to even minute quantities of allergens can lead to the production of IgE antibodies in atopic individuals.(Shamji *et al.*, 2021) . The total IgE levels are not associated with asthma severity.(helal *et al.*, 2020) .

1.2.9.2. Cytokines

A wide range of cytokines and chemokines may play important roles in asthma severity in pediatric patients(Hatami *et al.*, 2018) .Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells (Zhang and An, 2009) . A network of novel mediators known as 'pleiotropic cytokines' regulate the intermittent airway inflammation, bronchial smooth muscle hyper-reactivity and bronchoconstriction underlying asthma.(Jabbar AL-Hasnawi and AL-Hasnawi, 2020).

1.2.9.2. A. Interleukin 17

Interleukin-17 (IL-17, also known as IL-17A) is a key cytokine that links T cell activation to neutrophil mobilization and activation.(Saitoh *et al.*, 2009) . Although a signature cytokine of Th17 cells, IL-17 is now known to be expressed also by other adaptive and immune cell types, including CD8+ T cells, γδ T cells, natural killer T (NKT) cells, and innate lymphoid cells (Fig. 2).

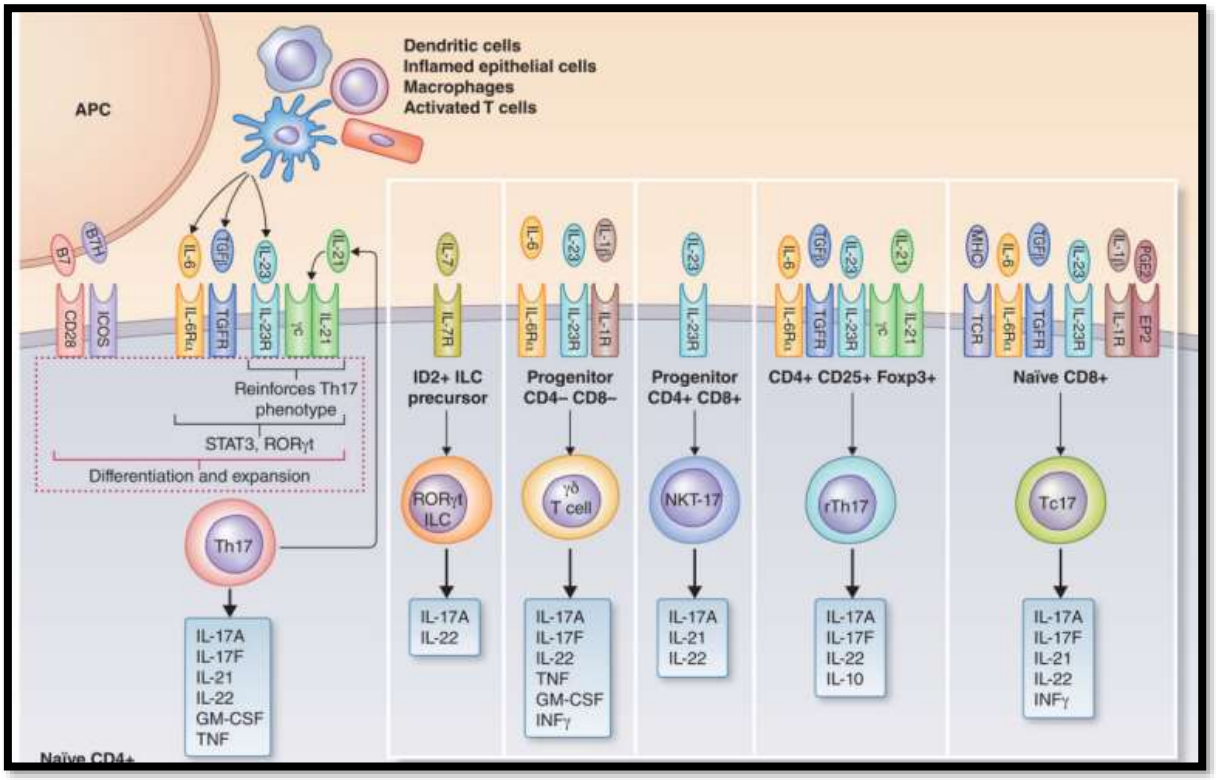


Figure 1.4: Differentiation of Th17 and other IL-17–producing cell subsets.(Rahmawati *et al.*, 2021)

Patients with higher levels of IL17 have a more severe asthma phenotype and biologics are available for T helper 2 (Th2)-high asthmatics .(Rahmawati *et al.*, 2021) .the interleukin 17 (IL-17) family of cytokines contains 6 structurally related cytokines, IL-17A through IL-17F. (McGeachy, Cua and Gaffen, 2019). This family is mainly involved in the host defense mechanisms against bacteria, fungi and helminth infection by inducing cytokines and chemokines, recruiting neutrophils, inducing anti-microbial proteins and modifying T-helper cell differentiation. (Chung, Ye and Iwakura, 2021).

The predominant roles of IL-17 cytokines are considered to be in maintaining the integrity of the epithelial surface barrier, and in responding to extracellular bacteria and fungi through the recruitment of neutrophils and the release of antimicrobial peptides (Hynes and Hinks, 2020). IL-17 have been demonstrate a wide range of receptors, including the neutrophil chemo-attractants CXCL1, CXCL5, CXCL8 (IL-8) and CCL2 ; granulocyte–macrophage colony-stimulating factor, which drives neutrophil production in the bone marrow .(Hynes and Hinks, 2020). And that correlating with disease severity and with sputum neutrophils. Certain studies suggested that IL-17 enhanced smooth muscle cell contractility with methacholine, and may drive hyper-responsiveness. (Hynes and Hinks, 2020) , While others suggesting that IL-17 stimulates human bronchial epithelial production of CCL28, which increases chemotaxis of IgE-containing B-cells, potentially important in allergic inflammation. IL-17 production is also increased in obesity-associated murine models of asthma and asthmatic pediatric patients. (Margelidon-Cozzolino *et al.*, 2022) .

1.2.9.2. B Interleukin-5

IL-5 is the most potent activator of eosinophils and is produced by Th2 cells and ILC2s. (Nagase, Ueki and Fujieda, 2020) .IL-5 is a cytokine known to play major role in the regulation of eosinophil formation, maturation, survival, and recruitment. Hence, an increased production of IL-5 may be contributed to the pathogenesis of asthma. (Takatsu, 2011) . . The expression of human IL-5

Receptor presented on eosinophils, basophils, and mast cells. Hence, a polymorphism in IL-5 receptor may be implicated in the development of asthma.(Pelaia, Busceti, *et al.*, 2020) . In asthmatic patients, IL-5 levels are elevated in the serum and the bronchoalveolar lavage fluid. (Takatsu, 2011) . Whoever IL-5 is also involved in a number of immune responses such as helminth infection and allergy.(Baldo *et al.*, 2023) .

1.2.10. Biomarkers of asthma

1.2.10.1. Periostin

Periostin has been identified as a molecule expressed in the osteoblast cell line MCT3- E1 and was initially named osteoblast-specific factor 2 (OSF-2). Subsequently, it was found to be highly expressed in the periosteum and periodontal ligament and was called periostin. Periostin is a member of the fasciclin family of proteins. Fasciclin family proteins have a FAS1 domain consisting of about 140 amino acids, a classic cell adhesiondomain maintained in various plants and animals. (Ono *et al.*, 2021).

Periostin is deeply involved in type-2 eosinophilic airway inflammation and remodeling in asthma. Several last studies approved that significantly higher level of serum periostin in asthmatics and in patients with uncontrolled compared to controlled asthma. It was significantly higher in those with severe and moderate asthma than those with mild asthma. Periostin level seems to be a strongly related factor for asthma and allergic rhinitis after 5 years of age. Serum periostin levels serve as a biomarker for both eosinophilic airway inflammation and fixed airflow limitation in well-controlled asthmatics on ICS treatment. Accumulating evidence shows that periostin, a matricellular protein, is involved in many fundamental biological processes such as cell proliferation, cell invasion, and angiogenesis.

In allergic inflammation response, periostin further amplifies the allergic inflammatory response (Figure 1-5). (Ono *et al.*, 2021) .

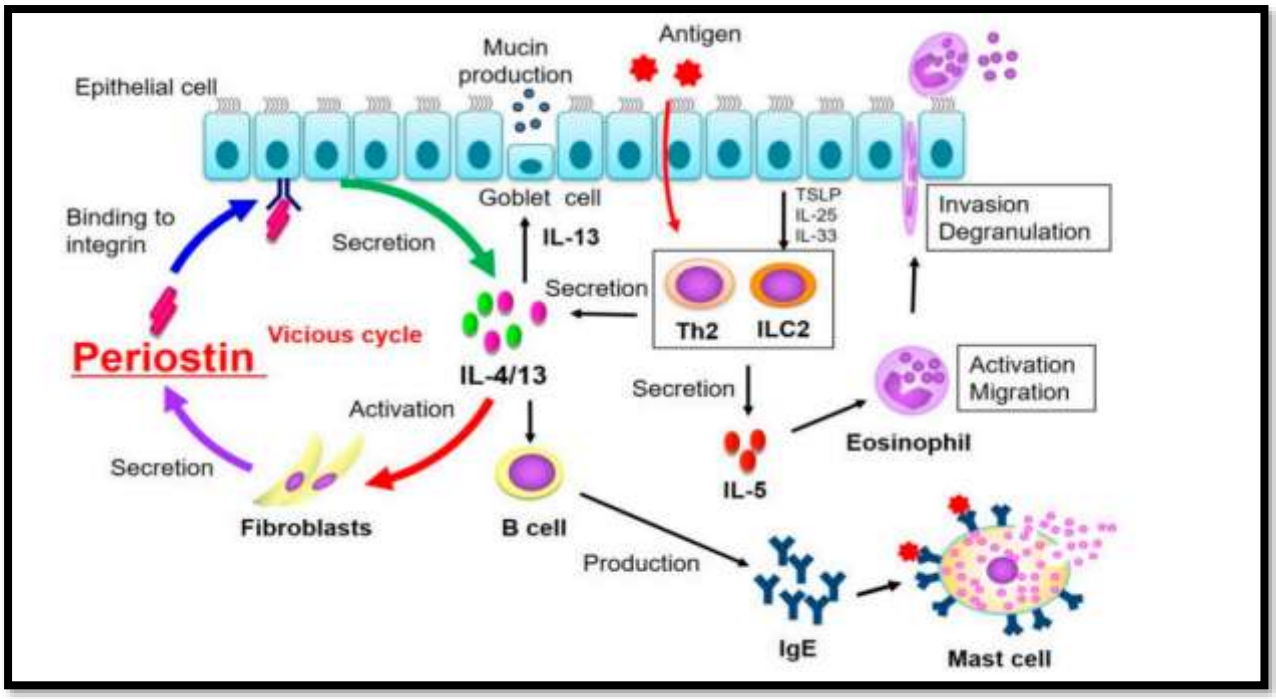


Figure 1.5: Summary of type 2 inflammatory response related to periostin in the pathogenesis of allergic diseases. (Ono *et al.*, 2021)

1.2.10.2. Serum Amyloid A1

Serum amyloid A (SAA) is, like C-reactive protein (CRP), an acute phase protein and can be used as a diagnostic, prognostic or therapy follow-up marker for many diseases. (De Buck *et al.*, 2016) .

In humans the major acute phase SAAs comprise a group of closely related variants of SAA1 and SAA2. SAA1 was proven to be chemotactic for several leukocyte subtypes through activation of the G protein-coupled receptor FPRL1/FPR2. (Abouelasrar Salama *et al.*, 2020) . SAA1 could induce the production of interleukin (IL)-6, IL-8, and S100 calcium-binding protein A9 from airway epithelial cells. (Bich *et al.*, 2022) . Additionally, SAA1 activated neutrophils and macrophages isolated from peripheral blood of asthmatics, releasing neutrophil extracellular traps (NETs) and secreting proinflammatory cytokines presenting Macrophage-1 phenotype. (Bich *et al.*, 2022) .It's also implicated to directly modulate innate and adaptive immune responses. (Ather *et al.*, 2018) .

The SAA1 levels were significantly higher in sera of asthmatic patients than in those of Healthy persons. SAA-high patients with asthma are characterized by systemic inflammation, and amplifies allergen-induced neutrophilic airway inflammation.

1.2.10.3. IL-4

IL-4 is a cytokine that functions as a potent regulator of immunity which secreted primarily by mast cells, Th2 cells, eosinophils and basophils. (Gadani, Sachin P; Cronk, 2013) . As a co-mitogen of B-cells, IL-4 was shown to be an important role in leukocyte survival under both physiological and pathological conditions such as, Th2 cell-mediated immunity, IgE class switching in B cells.

IL-4 is involved in T-cell differentiation, B-cell activation, B-cell differentiation into plasma cells, and the production of immunoglobulin E.(Nakagome and Nagata, 2024) .It is also consider as a central cytokines regulating allergic inflammation (Junttila, 2018). An additional mechanism by which IL-4 contributes to airway obstruction in asthma is through the induction of mucin gene expression and the hypersecretion of mucus. IL-4 increases the expression of eotaxin and other inflammatory cytokines from fibroblasts that might contribute to inflammation and lung remodelling in chronic asthma. An important activity of IL-4 in promoting cellular inflammation in the asthmatic lung is the induction of vascular cell adhesion molecule (VCAM)-1 on vascular endothelium. Through the interaction of VCAM-1, IL-4 is able to direct the migration of T lymphocytes, monocytes, basophils, and eosinophils to inflammatory loci.

In addition, IL-4 inhibits eosinophil apoptosis and promotes eosinophilic inflammation by inducing eosinophil chemotaxis and activation through the increased expression of Eotaxin. An essential biological activity of IL-4 in the development of allergic inflammation is the ability to drive the differentiation of naive T helper type 0 (TH0) lymphocytes into TH2 lymphocytes. These TH2 cells are able to secrete IL-4, IL-5, IL-9 and IL-13.

1.2.11. Clinical features of asthma

There are episodic or persistent symptoms of asthma such as dyspnea, chest tightness, wheezing, sputum production and cough, associated with variable airflow limitation and airway hyper-responsiveness to endogenous or exogenous stimuli. (Ducharme *et al.*, 2015). dyspnea is the main symptom of asthma, (Douros *et al.*, 2015) . Chest tightness is more specific to bronchoconstriction and is the earliest symptom of asthma (Douros *et al.*, 1996).

Wheezing disorders in childhood are common and vary, and various phenotypes have been proposed and classified by trigger (viral wheeze) and multiple trigger by other factors (van Aalderen, 2012) .also ,childhood wheeze can be linked to proposed mechanisms: allergic wheeze and non-allergic wheeze due to increased immune response to viral infection, or non-allergic wheeze due to structural airway narrowing. (van Aalderen, 2012) . In asthma, mucus hypersecretion is believed to result, at least in part, from inflammation, A variety of inflammatory mediators have been shown to stimulate mucus secretion including histamine, prostaglandins, leukotrienes, platelet activating factor, and eosinophil cationic protein .(Cohn *et al.*, 1997) .

While ,coughing in asthma has been attributed to reversible airflow obstruction and airway inflammation, the latter of which is typically eosinophilic (Niimi *et al.*, 2019) .in patients with nocturnal asthma there is a certain findings suggest that inflammation of the small airways contributes to asthma symptoms and the decrease in lung function at night. (Van Der Wiel *et al.*, 2013) .

1.2.12. Diagnostics hubs

There is no single ‘gold-standard’ test that can be used to accurately diagnose asthma.(Ullmann *et al.*, 2018). When asthma is correctly diagnosed, low-dose inhaled corticosteroids can easily control symptoms in most of patients. (Ullmann *et al.*, 2018) . In the absence of lung function tests, the diagnosis of asthma should be considered in children 1-5 years of age with frequent (≥8

days/month) asthma-like symptoms or recurrent (≥2) exacerbations (episodes with asthma-like signs).(Ducharme *et al.*, 2015) .

1.2.12. A. Airway inflammation measures:

1.2.12. A.1. Fractional Exhaled Nitric Oxide test (FeNO)

The FeNO concentration has moderate accuracy to diagnose asthma in individuals aged 5 years and older(Wang *et al.*, 2018) . FeNO testing as an option to help diagnose asthma in both children and adults when classic diagnostic tests for asthma have revealed an intermediate probability of having asthma. Nitric oxide is produced in the lungs as a result of eosinophilic inflammation, and can be detected in exhaled breath. FeNO is raised in many individuals with asthma, particularly atopic asthma.(Brigham and West, 2015) .

1.2.12. A.2. Lung function tests (Spirometry)

Spirometry is the most essential and commonly used lung function test. It is used mainly for the evaluation of lung function to obtain reliable data used for the detection of lung diseases, such as asthma, as well as for monitoring lung health. (Tony *et al.*, 2022) . Lung function tests can be used to aid the diagnosis of asthma in children over the age of 5 years.(Ullmann *et al.*, 2018) . Peak expiratory flow (PEF) and Forced expiratory volume in one second (FEV1) values can be used for diagnosing, monitoring, and determining the severity of asthma in patients, particularly in children.(Tony *et al.*, 2022) . Evidence of airway obstruction on spirometry, especially if reversible with a bronchodilator, can confirm the diagnosis of asthma or assess the severity. (Ullmann *et al.*, 2018) .

1.2.12. A.3. Spirometry parameters

1-Peak expiratory flow (PEF) or peak expiratory flow rate (PEFR) The measurement of the highest speed of expiration by using a spirometer device (in liter per second) or a peak flow meter device (in liter per minute) .

2-Forced expiratory volume in one second (FEV1) The volume of the air that is exhaled out of the lungs within the first second of forced expiration (occurs after extreme inhalation).

3-Forced vital capacity (FVC) The total amount of air that a person exhales as quick as possible while testing for FEV (occurs after maximum inhalation) .

4-FEV1/FVC ratio or FEV1% The percentage of FVC which is exhaled in one second .

5-Forced expiratory flow over the middle one-half of FVC (FEF25–75%) The average air flow from the level at which 25% of FVC has been expelled out to the level at which 75% of FVC has been expelled out .

6-Maximum voluntary ventilation (MVV) The total volume of air that an individual can breathe in and exhale within a definite period of time (Tony *et al.*, 2022) .

1.2.12. A.4. Peak Flow

The peak expiratory flow (PEF) is the maximal rate that a person can exhale during a short maximal expiratory effort after a full inspiration.(PEF-1) Measurements of peak expiratory flow are more variable and effort dependent. Thus, is more often used in the follow-up of patients to monitor patients' symptoms and response to therapy(Tony *et al.*, 2022). Peak flow variability, decrements in lung function and other biomarkers such as eosinophils may also increase during acute exacerbations and improve with recovery following treatment. (Wang *et al.*, 2021).

1.2.12. B. Airway hyperreactivity measures:

1.2.12. B.1. Bronchoprovocation Testing (BPTs)

There are two categories of bronchial provocation test, 'direct' and 'indirect'. The 'direct' BPTs include the pharmacological agent's histamine and methacholine. These agents cause airway narrowing by acting 'directly' on their

respective receptors on bronchial smooth muscle and cause airway constriction.(Leuppi, 2014). While 'indirect' challenge tests include the physical stimuli, such as exercise, dry air hyperpnea, distilled water, hypertonic saline and mannitol. These stimuli are thought to cause airway narrowing 'indirectly' by releasing a wide variety of mediators of bronchoconstriction from inflammatory cells within the airways. These mediators then act on specific receptors on bronchial smooth muscle cells to cause airway narrowing. (Leuppi, 2014) .

1.2.12. B.2. Other tests: Allergy Testing

Allergy testing can be performed by skin testing or by specific immunoglobulin E (IgE) in serum. Skin testing is simple to perform on a wide variety of common environmental allergens (Brigham and West, 2015) . Serum testing, though can be the better choice in a patient with a documented anaphylaxis history, it is then important to confirm that the patient's exposure to the allergen does cause the asthmatic symptoms. (Brigham and West, 2015).

1.2.13. Treatment of asthma

Treatment of a chronic disease such as asthma involves different aspects, and recommendations must be oriented individually.(Bertrand, 2020).

Introduction of inhaled corticosteroids (ICS) has been the cornerstone of the long-term management of asthma. ICSs either alone or in combination with long-acting beta-2 agonists have been shown to be associated with favorable asthma outcomes. (Çelik *et al.*, 2023) . Asthma treatment is not "one size fits all" (Levy *et al.*, 2023) .the pharmacological options for treatment of asthma include, , reliever medications, which are drugs that allow relief of symptoms within few minutes, and controller medications, that are used for maintenance treatment: they control symptoms and reduce airway inflammation (Tesse *et al.*, 2018) .

1.2.13. A. Medications Used for Rapid Relief of Symptoms

At present, step-1 treatment is with as-needed inhaled short-acting beta2-agonists (SABAs) alone, commonly salbutamol. SABAs are used for acute relief of asthma symptoms, (Tesse *et al.*, 2018) .

1.2.13. B. Medications Used for Long-Term Asthma Symptoms Control Inhaled Corticosteroids (ICS)

More frequent symptoms indicate that regular controller treatment is needed. For long term asthma control in children, a maintenance treatment with therapeutic doses of ICS in addition to as-needed SABA, should be considered. (Tesse *et al.*, 2018) .

1.2.13. C. Long-Acting Beta-2 Adrenergic Agonists (LABA)

the combination of ICS and LABA, including salmeterol and formeterol, improves asthma outcomes more than higher doses of ICS (Tesse *et al.*, 2018).

1.2.13. D. Leukotriene Receptor Antagonists (LTRAs)

LTRAs work by blocking some inflammatory responses, such as tightening of airway muscles and the production of mucus secretion, mediated by leukotriens, which are released during asthmatic reaction (Tesse *et al.*, 2018).

1.2.14. Immunotherapy for asthma

Allergen specific immunotherapy (AIT) is the only causal therapeutic option for allergic airway diseases including asthma and allergic rhinitis. (Diamant *et al.*, 2023) .the introduction of biologics for the treatment of patients with refractory asthma represented a marked therapeutic advance.(Ridolo *et al.*, 2020) .

1.2.15. Role of biologic therapy in asthma

IL-5 and its receptor are suitable targets for selective biologic drugs, the anti-IL-5 monoclonal antibodies mepolizumab and reslizumab have been developed and approved for biological therapy of uncontrolled eosinophilic asthma.(Pelaia *et al.*, 2018). Due to the critical role that IL-5 plays in all areas of eosinophil activity, it has been identified and targeted by several therapeutics.

1.2.15.1. Omalizumab

Is an anti-IgE humanized monoclonal antibody which is use in moderate-to-severe persistent asthma in allergic adults and children over 6 y old (Ridolo *et al.*, 2020) . The real-life therapeutic effectiveness of omalizumab coexists with a long-term, very good safety and tolerability profile (Zhang *et al.*, 2023) . omalizumab inhibits all IgE-dependent cellular and molecular events involved in

the immune/ inflammatory cascade underlying allergic asthma. (Pelaia, Crimi, *et al.*, 2020) .

1.2.15.2. Mepolizumab

Is a humanized monoclonal antibody (mAb) against IL-5 which selectively inhibits eosinophilic inflammation and reduces the amount of eosinophils. (Hynes and Hinks, 2020) . Mepolizumab, one of the earliest licensed anti-IL-5 biologic, has been demonstrated to patients with severe eosinophilic asthma.(Morjaria *et al.*, 2019) . Mepolizumab specifically binds to the α-chain of IL-5 which effectively inhibits IL-5 ligation to IL-5Rα. (Pelaia *et al.*, 2017).

1.2.15.3. Benralizumab

Is a humanized, fucosylated, monoclonal antibody that targets the interleukin 5 (IL-5) α receptor, which can significantly reduce the incidence of acute exacerbations and improve lung function in patients with severe asthma.(Izumo *et al.*, 2020). the clinical effects in reducing asthma exacerbations and in improving the quality of life and lung function are clear (Padilla Galo *et al.*, 2018)

1.2.15.4. Secukinumab

Is a human IgG1k monoclonal antibody targeting IL-17A. (Bush, 2019). It had been evaluated in the ozone-induced airway neutrophilic inflammation (Hayes *et al.*, 2020). It has been tested in patients with uncontrolled asthma (Cazzola *et al.*, 2022) .

1.2.16. Control of asthma

The Global Initiative for Asthma (GINA) proposes to distinguish between controlled, partly controlled and poorly controlled asthma (Lødrup Carlsen *et al.*, 2015) .control of asthma can be achieved by avoidance of triggers, patient adherence to controller medication, and the patient's ability to recognize asthma symptoms.(Murray and O'Neill, 2018) . Despite widespread focus on control as the goal of asthma treatment, relatively large proportions of patients around the world are poorly controlled (Vernon *et al.*, 2013) . Asthma self-management education is essential to the control of asthma. (Murray and O'Neill, 2018), which

is done by twofold: to raise awareness of effective inhaler technique and to support self-management of asthma triggers for health and symptom control.(Murray and O'Neill, 2018) .

However, asthma education significantly decreased number of patients requiring emergency room visits and hospital admissions. (Mishra *et al.*, 2017) . Allergy Trigger Avoidance,] offering suggestions such as staying indoors during mid-day and afternoon hours when pollen counts are highest for pollen allergies (Bridgeman and Wilken, 2021), avoiding dried flowers which may contain mold, washing hands after handling pets for pet allergies, along with a number of other tips for ways to reduce exposure to common allergic asthma triggers. (Vernon *et al.*, 2013) . Information on triggers plays a central role in the diagnosis and control of asthma (Vernon *et al.*, 2013) . Allergen avoidance measures have been widely utilized; however, there is ongoing controversy on the effectiveness of specific allergen control measures in the control of pediatric asthma.(Kalayci *et al.*, 2022).

Despite the availability of therapies, asthma control remains suboptimal due to low adherence, poor inhaler technique. Proper inhalation technique and medication compliance are essential factors and indicators for successful asthma treatment (Khdour *et al.*, 2020).

Chapter Two

Materials and Methods

2. Material and Methods:

2.1. Study Design: A cross-sectional study.

2.2. Patients

One hundred asthmatic patients enrolled who have been diagnosed by specialist pulmonologist in the pulmonary centre of Kerbala Teaching Hospital for Children. The study was extended from October 2023 to July 2024. All of the One hundred were on treatment with either inhaled corticosteroids or Montelukast. The age's range of the patients was (5-15) and the male proportion (75%) and female proportion was (25%).

2.2.1. A. grouping of patients: patients are classified into 4 groups:

Group1: Eosinophilic group: include patients with increased blood eosinophil counts >3%. (Hirano and Matsunaga, 2023)

Group2: Neutrophilic group: include patients with increased blood neutrophil counts >5% **.**(Flinkman *et al.*, 2023)

Group3: Mixed granulocytic groups: include these patients which records high counts of both eosinophil and neutrophils. (Flinkman *et al.*, 2023)

Group4: Allergic groups: include these patients which report positive Specific IgE inhalation and record high counts of eosinophil.(Zhang *et al.*, 2023)

2.2.2. Inclusion and Exclusion Criteria:

2.2.2.1. Inclusion criteria:

- Child above five years old; both sexes, those had diagnosed as asthma.
- Patients on inhaled corticosteroids are included.

2.2.2.2. Exclusion criteria:

- Patients were excluded whose have biological therapy and systemic corticosteroid drugs.
- All patients' upper and lower respiratory infection diseases.
- Patients with autoimmune disease and these with cystic fibrosis also should be excluded.

2.2.2.3. Ethical approval: The study protocol sent to the ethical committee of Karbala College of Medicine & the relevant ethical committee in Kerbala health directorate. In addition, verbal approval taken from the patient's relatives before taking the sample. Health measures and safety taken during sampling.

2.2.2.4. Patient data: Demographic and Clinical data collected using a specific detailed questionnaire, were designed to search from Kerbala pulmonary centre in Kerbala Teaching Hospital for Children, and the Research Committee, taking the international and local standards into account, for collecting data from children with asthma

2.3. Sample Collection:

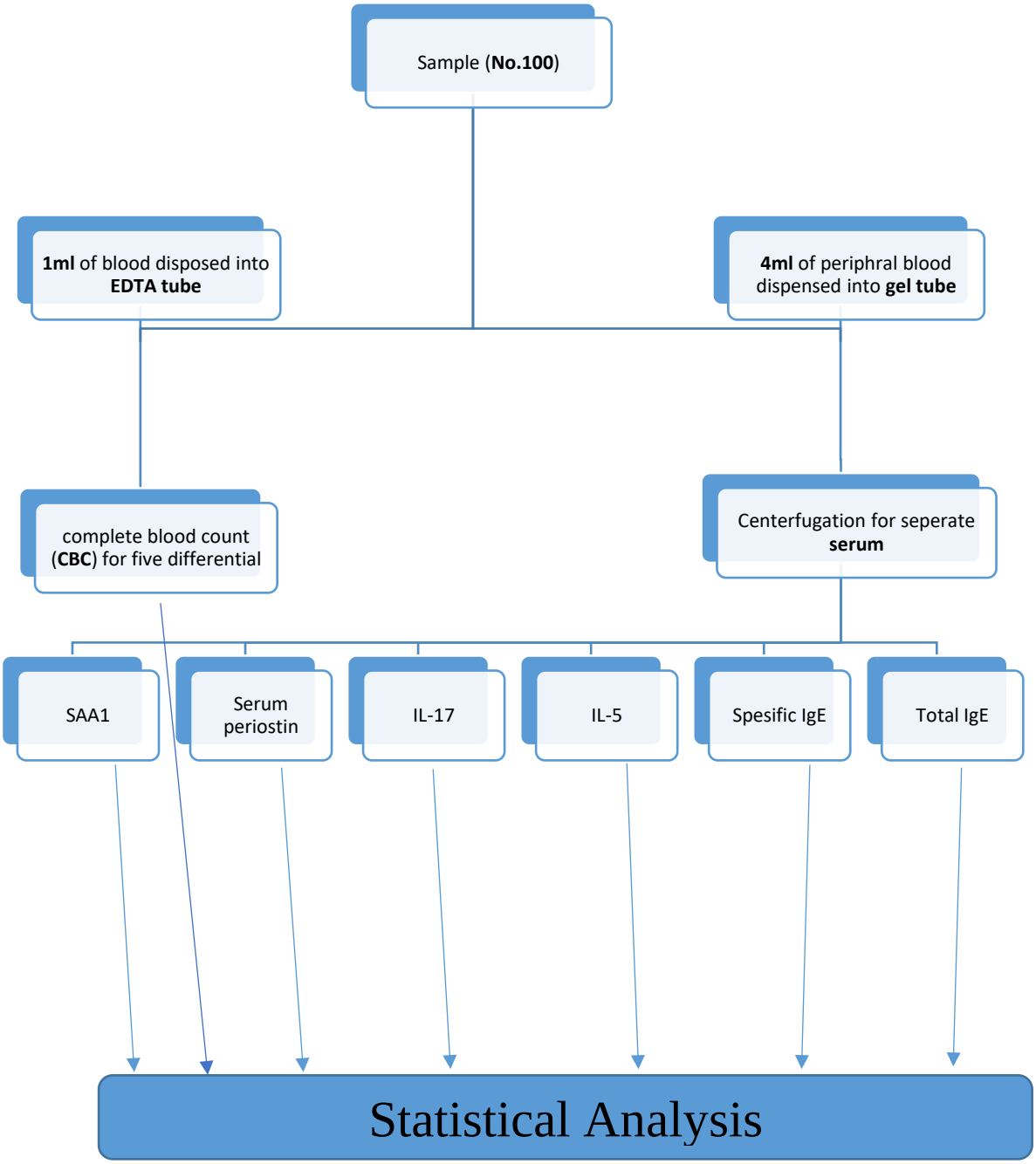
Approximately 5 ml of venous blood were drawn from each subjects which were obtained by disinfecting antecubital fossa with 70% ethanol and then make vein puncture by disposal syringes after applying a tourniquet. One ml of blood were dispensed into (1) EDTA tube for the haematological tests. Four ml of blood were dispensed into gel tube and allowed to clot then serum was separated by centrifugation at 3000 round per minutes (RPM) for 5 minutes. Then the serum was transferred to new appendorff tube (2 ml) and stored in deep freeze (-20°C) to be used for immunological assays.

2.3.1. Serology tests:

ELISA technique for SAA1, IL-17, IL-5, priostin and Total IgE, while Specific Aeroallergens inhalation detection is done by micro array of assay strips (EUOROIMMUNE).

2.3.2. Schema of study design:

The design of study was illustrated in Figure (2-1).



2.4. Materials:

2.4.1. Equipment and Instruments:

In the present study, the following Equipment and Instruments were used:

Table (2-1): Equipment and Instruments used in study.

No.	Equipment & Instruments	Company/Origin
1	Appendroff tube(1.5 ml)	Medicho(China)
2	Centrifuge	Rotofix 32 A (Germany)
3	Cold medical box	Cool ice box Co.(UK)
4	Cotton	Medicho(China)
5	Different size of tip	Medicho (China)
6	Different size pippete	Hawach (China)
7	EDTA tube	SKgmed (China)
8	ELISA DEIGE02	Demeditec, Germany
9	Elisa reader	HumaReader HS human (Germany)
10	Elisa washer	Combiwash human (Germany)
11	Freezer	Concord (China)
12	Gel tube	SKgmed (China)
13	Gloves	Medstock (Australia)
14	Ice back	SKgmed (China)
15	Mechanical pippite	Hawach (China)
16	Multiple mechanical pippete	Hawach (China)
17	Rack	SKgmed (China)
18	Refrigerator	LG(Korea)
19	Rocking mixer	Euroimmune
20	Shaker	Heidolph reax top (Germany)
21	Syringe	Medicho (China)
22	Sysmex XN-350	Sysmex, Japan
23	Thermo-shcker	Biosan (Latvia)
24	Tornicha	Medicho (China)
25	Zibio hematology analyzer	Zybio clinic Co.(China)

2.4.2. ELISA Kit:

Table 2.2: ELISA Kits used in the study

No.	ELISA Kit	Manufacturing Company/orign
1-	Human Interleukin 17	Bioassay technology laboratory (China)
2-	Human Interleukin 5	Bioassay technology laboratory (China)
3-	Human Periostin	Bioassay technology laboratory (China)
4-	Human Serum amyloid A1	Bioassay technology laboratory (China)
5-	Total IgE	Demeditec Diagnostics GmbH (Germany)

2.4.2.1. ELISA Kit Content of Total Serum IgE:

Table (2-3): ELISA kit for detection of human total serum IgE

No.	**Components**	**Format**
1-	Monoclonal anti-IgE coated microtiter strips	12×8
2-	Calibrators (Standards)(0, 5, 25, 100,250,1000 IU/mL)	1x 1 mL; 5x 200 µL
3-	Enzyme Conjugate (goat anti-IgE-HRP)	22 ml
4-	Substrate	12 ml
5-	Stop Solution	12 ml
6-	Washing Buffer (10×)	60 ml

2.4.2.2. ELISA Kit Content of human interleukin 5 (IL-5):

Table (2-4): ELISA kit for detection of human interleukin 5 (IL-5)

No.	Components	Quantity
1-	Standard Solution (960ng/L)	0.5ml x1
2-	Pre-coated ELISA Plate	12 * 8 well strips x1
3-	Standard Diluent	3ml x1
4-	Streptavidin-HRP	6ml x1
5-	Stop Solution	6ml x1
6-	Substrate Solution A	6ml x1
7-	Substrate Solution B	6ml x1
8-	Wash Buffer Concentrate (25x)	20ml x1
9-	Biotinylated Human IL-5 Antibody	1ml x1
10-	User Instruction	1
11-	Plate Sealer	2 pics
12-	Zipper bag	1 pic

2.4.2.3. ELISA Kit Content of human interleukin 17 (IL-17)

Table (2-5): ELISA kit for detection of human interleukin 17 (IL-17)

No.	Components	Quantity
1-	Standard solution (640ng/L)	0.5ml x1
2-	Pre-coated ELISA plate	12 * 8 well strips x1
3-	Standard diluent	3ml x1
4-	Streptavidin-HRP	6ml x1
5-	Stop solution	6ml x1
6-	Substrate solution A	6ml x1
7-	Substrate solution B	6ml x1
8-	Wash buffer Concentrate (25x)	20ml x1
9-	Biotinylated Human IL17A antibody	1ml x1
10	User instruction	1
11-	Plate sealer	2 pics

2.4.2.4. ELISA Kit Content of human serum periostin (POSTIN):

Table (2-6): ELISA kit for detection of human serum periostin (POSTIN):

No.	Components	Quantity
1-	Standard solution (192ng/ml)	0.5ml x1
2-	Pre-coated ELISA plate	12 * 8 well strips x1
3-	Standard diluent	3ml x1
4-	Streptavidin-HRP	6ml x1
5-	Stop solution	6ml x1
6-	Substrate solution A	6ml x1
7-	Substrate solution B	6ml x1
8-	Wash buffer Concentrate (25x)	20ml x1
9-	Biotinylated Human serum priostin antibody	1ml x1
10	User instruction	1
11-	Plate sealer	2 pics

2.4.2.5. ELISA Kit Content of human serum amyloid A1 (SAA1)

Table (2-7): ELISA kit for detection of human serum amyloid A1 (SAA1)

No.	Components	Quantity
1-	Standard Solution (40ug/ml)	0.5ml x1
2-	Pre-coated ELISA plate	12 * 8 well strips x1
3-	Standard diluent	3ml x1
4-	Streptavidin-HRP	6ml x1
5-	Stop solution	6ml x1
6-	Substrate solution A	6ml x1
7-	Substrate solution B	6ml x1
8-	Wash buffer Concentrate (25x)	20ml x1
9-	Biotinylated Human SAA Antibody	1ml x1
10	User instruction	1
11-	Plate sealer	2 pics

2.4.2.6. Inhalation specific IgE Kit:

Table (2-8) Contents of a test Inhalation specific IgE kit:

No.	**Description**	**Format**
1-	Test strips coated with the allergens	16 strips
2-	Enzyme conjugate : Alkaline phosphatase labelled anti-human IgE (mouse)	1x30ml
3-	Universal buffer : 10x concentrated	1x100ml
4-	Substrate solution : Nitroblue tetrazolium chloride / 5-bromo-4-chloro-3-indolyphosphate (NBT/BCIP), ready for use	1x30ml
5-	Incubation tray: volume reduced(400) micro-litter	2x10 channels

2.5. Methods:

2.5.1. Complete Blood Count:

The blood specimen in EDTA tubes was shaken up then was examined as soon as possible in Sysmex XN-350 five differential automated haematology analyzer (Sysmex, Japan) to count white blood cells.

2.5.2. ELISA

2.5.2.1. Measurement of Total Serum IgE:

Serum was analysed to determine the total IgE concentration by Demeditec Total IgE ELISA DEIGE02 automated immunoassay analyzer (Demeditec, Germany) using Demeditec total IgE ELISA kit (DEIGE02).

2.5.2.1.1. Principle of the test:

The Total IgE ELISA is based on the principle of the enzyme immunoassay (EIA). A monoclonal mouse-anti-human IgE antibody is bound on the surface of the microtiter strips. Undiluted patient serum was pipetted into the wells of the microtiter plate together with antihuman-IgE-peroxidase conjugate.

A sandwich complex between the serum IgE and the two antibodies develops. After a 30 minutes´ incubation at room temperature, the plate was rinsed with diluted wash solution, in order to remove unbound material. Then the substrate (TMB) solution was pipetted and incubated for 15 minutes, inducing the development of a blue dye in the wells. The colour development was terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye was measured spectrophoto metrically at the wavelength of 450 nm. The concentration of the IgE antibodies is directly proportional to the intensity of the colour.

2.5.2.1.2. Procedure of test:

1. All reagents were prepared prior to starting test procedure. All standards and samples were added into duplicate to the micro ELISA strip plate.
2. 10 µL each of the undiluted samples and 200 µL standards together conjugate into the wells. While one well empty for the substrate blank.
3. The plate then cover and incubate at room temperature for 30 minutes.
4. All the wells of the plate are Empty (dump or aspirate) and then 300 µL of diluted washing solution is added.
5. 100 µL of substrate was Pipetted into the wells.
6. The plate was Cover and incubate at room temperature for 15 minutes in the dark (e.g. drawer).
7. 100 µL of stop solution was Pipetted into the wells.
8. Then mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm

2.5.2.1.3. Result Interpretation:

Table (2-9) Normal ranges of Total IgE (Carosso *et al.*, 2007)

Age	Normal Range [IU/mL]
6 – 9 years	< 155
10 – 15 years	< 199

2.5.3. Measurement of human serum IL-17 antibody:

2.5.3.1. Principle of the test:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate had been pre-coated with Human IL17A antibody. IL17A present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human IL17A Antibody was added and binds to IL17A in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated IL17A antibody.

After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human IL17A. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

2.5.3.2. Procedure of test:

1. All reagents, standard solutions and samples were Prepared and performed at room temperature.

2. 50ul standard was added to standard well, then

3. 40ul of the sample was added to sample wells and then 10ul Human IL17A antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells, then mixed well. Then the plate was covered with a sealer. And incubate 60 minutes at 37°C.

4. The plate was washed 5 times with wash buffer for 30 seconds to 1 minute for each wash.

5. 50ul substrate solution A was added to each well and then add 50ul substrate solution B was added to each well. The plate was covered and Incubated for 10 minutes at 37°C in the dark.

6. Then 50ul of the Stop Solution was added to each well, the blue color will change into yellow immediately.

7. Each well was Determined the optical density (OD value) immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5.4. Measurement of human serum IL-5 antibody:

2.5.4.1. Principle of the test:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate had been pre-coated with Human IL-5 antibody. IL-5 present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human IL-5 Antibody was added and binds to IL-5 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated IL-5 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human IL-5. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

2.5.4.2. Procedure of test:

1. All reagents, standard solutions and samples were Prepared and performed at room temperature.

2. 50ul standard was added to standard well, then

3. 40ul of the sample was added to sample wells and then 10ul Human IL-5 antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells, then mixed well. Then the plate was covered with a sealer. And incubate 60 minutes at 37°C.

4. The plate was washed 5 times with wash buffer for 30 seconds to 1 minute for each wash.

5. 50ul substrate solution A was added to each well and then add 50ul substrate solution B was added to each well. The plate was covered and Incubated for 10 minutes at 37°C in the dark.

6. Then 50ul of the Stop Solution was added to each well, the blue color will change into yellow immediately.

7. Each well was Determined the optical density (OD value) immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5.5. Measurement of human serum priostin (POSTN) antibody:

2.5.5.1. Principle of the test:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate had been pre-coated with Human priostin (POSTN) antibody. Priostin (POSTN) present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human priostin (POSTN) Antibody was added and binds to priostin (POSTN) in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated priostin (POSTN) antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human priostin (POSTN). The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

2.5.5.2. Procedure of test:

1. All reagents, standard solutions and samples were Prepared and performed at room temperature.

2. 50ul standard was added to standard well, then

3. 40ul of the sample was added to sample wells and then 10ul Human priostin (POSTN) antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells, then mixed well. Then the plate was covered with a sealer. And incubate 60 minutes at 37°C.

4. The plate was washed 5 times with wash buffer for 30 seconds to 1 minute for each wash.

5. 50ul substrate solution A was added to each well and then add 50ul substrate solution B was added to each well. The plate was covered and Incubated for 10 minutes at 37°C in the dark.

6. Then 50ul of the Stop Solution was added to each well, the blue color will change into yellow immediately.

7. Each well was Determined the optical density (OD value) immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5.6. Measurement of human serum Amyloid A1 antibody:

2.5.6.1. Principle of the test:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate had been pre-coated with Human SAA antibody. SAA present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human SAA Antibody was added and binds to SAA in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated SAA antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human SAA. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

2.5.6.2. Procedure of test:

1. All reagents, standard solutions and samples were Prepared and performed at room temperature.

2. 50ul standard was added to standard well, then

3. 40ul of the sample was added to sample wells and then 10ul Human SAA antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells, then mixed well. Then the plate was covered with a sealer. And incubate 60 minutes at 37°C.

4. The plate was washed 5 times with wash buffer for 30 seconds to 1 minute for each wash.

5. 50ul substrate solution A was added to each well and then add 50ul substrate solution B was added to each well. The plate was covered and Incubated for 10 minutes at 37°C in the dark.

6. Then 50ul of the Stop Solution was added to each well, the blue color will change into yellow immediately.

7. Each well was Determined the optical density (OD value) immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5.7. Inhalation Specific IgE test

2.5.7.1. Principle of the test:

2.5.7.1. a. Preparation and stability of the sample:

2.5.7.1.1. Stability:

The sample was prepare, by dilute 100 µl of serum with 1.0 ml working-strength universal buffer and mix thoroughly by vortexing , The final volume will be 1.1 ml of (diluted sample).

2.5.7.1.2. Incubation:

2.5.7.1.2. a. Pre-treat:

The test strips are incubated in incubation tray, then each of the channels was filled with 1ml of working-strength universal buffer and incubate the test strips for (5 minutes) at room temperature (+18°C to +25°C) 0n a rocking shaker (20 to 50 rpm). Afterwards aspirate off all the liquid.

2.5.7.1.3. Sample incubation (1st step)

Each channels in incubation tray was filled with 1.1 ml of (diluted sample), then incubated overnight (12h to 24h) on a rocking shaker (20 to 50 rpm) at room temperature with cover the incubation tray for prevention of evaporation.

2.5.7.1.3. A. Washing:

The liquid from each channel was a aspirated off and wash for 3 x 5 minutes with 1.0 ml of working-strength universal buffer on a rocking shaker (20 to 50 rpm).

2.5.7.1.3.B. Conjugate incubation (2nd step):

1ml of the enzyme conjugate(Alkaline phosphatase labelled anti-human IgE) was transferred to each channel and incubated for 60 minutes at room temperature (+18°C to +25°C) on a racking shaker (20 to 50 rpm).

2.5.7.1.3. C. Washing:

The liquid from each channel is Aspirate off and wash for 3 x 5 minutes with 1.0 ml of working-strength universal buffer on a rocking shaker (20 to 50 rpm).

2.5.7.1.3.D. Substrate incubation (3rd step):

1ml of the substrate was transfer to each channel and incubate for 10 minutes at room temperature (+18°C to +25°C) on a racking shaker (20 to 50 rpm).

2.5.7.1.3. E. Stopping:

The liquid of each channel was aspirated and wash each strips 3 x 1 minute with deionised or distilled water.

2.5.7.1.3. F. Evaluate:

The test strips are places on the evaluation protocol, air dry and evaluate.

2.5.8. Short protocol for test:

Pre-treatment

- Insertion of test strip in an incubation channel
- Addition of 1.0 ml of working-strength universal buffer by pipetting

5 min rocking shaker

1. Sample incubation:

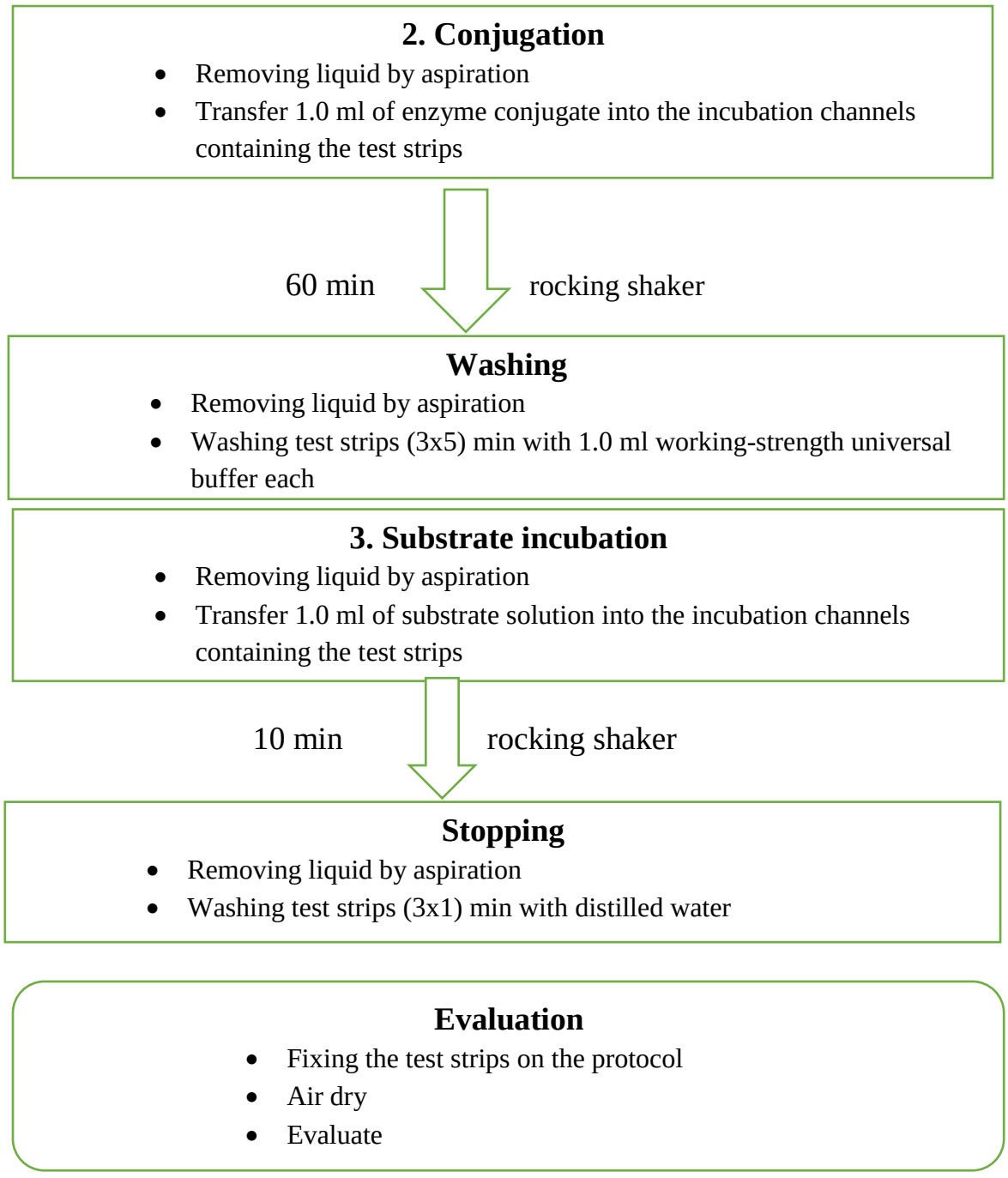
- Remove liquid by aspiration
- Pipette samples into the tray channels

- **1000 µl** working-strength universal buffer
- **100 µl** sample

12-24h rocking shaker

Washing

- Removing liquid by aspiration
- Washing test strips (3x5) min with 1.0 ml working-strength universal



2.5.9. Interpretation of results

After stopping the reaction by using distilled water, all test strips are pressed on filter paper and left to dry without any direct light. The test strips are only take place after

the strips are completely dried, then the evaluation of those strips are done by the EURO Line scan program.

Table (2-10) the classes can be divided into the following concentrations:

Class	Concentration[kU/I]	Result
0	< 0.35	No specific antibodies detected.
1-	0.35 ≤ sIgE < 0.7	Very low antibody titer, frequently no clinical symptoms where sensitization is present.
2-	0.7 ≤ sIgE < 3.5	Low antibody titer, existing sensitization, frequently with clinical symptoms in the upper range of class present.
3-	3.5 ≤ sIgE < 17.5	Significant antibody titer, clinical symptoms usually present.
4-	17.5 ≤ sIgE < 50.0	High antibody titer, almost always with clinical symptoms.
5-	50.0 ≤ sIgE < 100.0	Very high antibody titer.

2.5.10. Statistical analysis

The data analysis for present investigation was generated using The Statistical Package for the Social Sciences software, version 26 (IBM, SPSS, and Chicago, Illinois, USA). Descriptive statistics was performed on the patient's data of each group. Data was analyzed for means, and the standard deviation was computed for the continuous variables, whereas frequency was used for computing the qualitative data. The mean of the investigated biomarkers were compared between the studied groups using t-Test; Chi-Square analysis was employed to significant compare between percentages; Differences among groups were analyzed using one-way ANOVA analysis of variance. Also, Pearson correlation coefficients were calculated to check the relationship between the studied markers. All hypothesis test results with two-sided p-values less than 0.05 were deemed statistically significant. (Duncan et al., 1983; Basher, 2003)

Chapter Three

Result

3. The results

3.1. Demographic date and patients' classification

The table (3-1) showed the demographic data of asthmatic patients were included in current study, when age, sex, residency, passive smoking, family history, Food allergy, Allergic Rhinitis, and Pets at home. The highest percent (59.0%) of patients within the age group 9-12 y, and the Mean ± SD were 9.71 ± 2.328

The majority (75%) of asthma patients were male, while only (25%) were female. In the context of residency, the highest percent (65%) of patients were resided in urban, while only (35%) resided in rural. As for passive smoking, patients were divided into two groups: passive smoking were (48%) and non-passive smoking were (52%).

Regarding family history, most patients (70) % had a family history of asthma, while only (30) % had no family history of the disease. As for food allergy, patients were divided into two groups according to present or absent food allergy. The results of the statistical analysis showed that the majority (82%) of patients in the current study did not have food allergy, while the smaller percentage (18%) had a food allergy. with respect to Allergic Rhinitis these patients with allergic rhinitis were (57%), while (43%) were non-allergic, and the patients whose have a pet at home, were (52%), while those does not have were (48%).

Regarding to CBC counts, the asthmatics patients was classify into; (65%) of patient had eosinophilic asthma, while (32%) had neutrophilic asthma, whilst those with mix granulocytic asthma were (30%), and those patients with allergic asthma were (22%).

Table (3-1) Demographic date and patients' classification

Category		Count	%
Age (9-12) year, Mean ±SD (7.91±2.328)		59	59%
Gender	Male	75	75.0%
	Female	25	25.0%
Residence	Urban	65	65.0%
	Rural	35	35.0%
Passive smoke	Present	48	48%
	Absent	52	52%
Family history	Yes	70	70.0%
	No	30	30.0%
Food allergy	Yes	18	18.0%
	No	82	82.0%
Allergic rhinitis	Yes	57	57.0%
	No	43	43.0%
Pets at home	Yes	52	52.0%
	No	48	48.0%
Type of asthma	Eosinophilic	65	65.0%
	Neutrophilic	32	32.0%
	Mixed granulocytic	30	30.0%
	Allergic	22	22.0%

3.2. Classification of asthma patients according studied parameters:

The table (3-2) showed Patients were classified according to the studied parameters, which included: Lymphocyte count, Neutrophil count, Eosinophil count, mixed granulocytic count and allergic count (those patients with high eosinophilia

and positive Aeroallergen (Specific IgE inhalation), FeNo level, total IgE (T. IgE) level, Specific IgE inhalation.

Regarding to Lymphocyte count, Neutrophil count, and Eosinophil count, patients were divided into three groups (High, Low and Normal) according to the count of these parameters in asthma patients. The majority (78%) of patients had normal count of Lymphocyte, while the (32%) of those patients had high Neutrophil count, as for Eosinophil count, patients with high eosinophil count were higher (65%) than those with low (14%) and normal count(21%).

In the context of mixed granulocytic group**,** patients were divided into two groups: mix-group and non-mix-group, the percent of patients within no-mix-group were higher (70%) than those within mix-group (30%). In the other hand, allergic group, patients were divided into groups allergic and non-allergic. (78%) of the patients had non allergic, while the other were (22%) allergic. The normal levels of FeNo were (83%), compared with those with high levels, while (17%) with high levels of FeNo. With respect to T. IgE level, where patients were divided according to its concentration into only two groups: Normal and Abnormal; patients with abnormal T. IgE level were higher (32%) than those with normal T. IgE level (68%). As for S. IgE, the percent of patients were positive (54%) to S. IgE higher than those patients were negative to S. IgE, (46%).

Table (3-2): Classification of patients according to studied parameters

Lymphocyte Count				
Range	**High** > 3.2*10^9/L	**Low** < 1.1*10^9/L	**Normal** 1.1 ~ 3.2*10^9/L	Total
Count (%)	15 (15%)	7 (7%)	78 (78%)	100
Neutrophil Count				
Range	**High** 5.5 > *10^9/L	**Low** 1.8 < *10^9/L	**Normal** 1.8 ~ 5.5*10^9/L	Total
Count (%)	32 (32%)	34 (34%)	34 (34%)	100
Eosinophil Count				
Range	**High** > 0.03 *10^9/L	**Low** < 0.02*10^9/L	**Normal** 0.02~0.0 *10^9/L	Total
Count (%)	65 (65%)	14 (14%)	21 (21%)	100
Allergic Count [S.IgE+ & Eosin > 0.03*10^9]				
	Allergic	Non-Allergic		Total
Count (%)	22(22%)	78 (78%)		100
Mixed granulocytic Count (High Eosin & Neut)				
Range	Mix	Non-Mix	Total	
Count (%)	30 (30%)	70 (70%)	100	
FeNo Level <20 ~ >35 (ppb)				
Range	High >35 (ppb)	Normal **(**< 20 ~ > 35 ppb**)**		Total
Count (%)	17 (17%)	83 (83%)		100
Total IgE level (155~199) [IU/mL]				
Range	High >199	Normal (155 ~199)	Total	
Count (%)	32 (32%)	68 (68%)	100	
Specific IgE Count				
	Positive	Negative	Total	
Count (%)	54 (54%)	46 (46%)	100	

3.3. Biomarkers concentrations in asthmatic patients

3.3.1. According to Eosinophil and Neutrophil groups (counts):

The table (3-3) show the concentrations of studied markers (SAA1, IL-17, IL-5, and Periostin), which displays according to Eosinophil and neutrophil count in

asthmatic patients, which is divided into three groups: high, low and normal. Statistical analysis using one way-ANOVA test showed non-significant (p>0.05) differences in the distributions of SAA1 and IL-17, while highly significant (P-value =0.0015 and P-value =0.0054 respectively) for IL-5 and priostin, in eosinophilic group. While SAA1 and IL-17 revealed highly significant in neutrophilic group (P-value=0.0064 and P-value=0.0072 respectively).

Table (3-3): Biomarkers concentrations in asthmatic patients according to Eosinophil and neutrophil count

Biomarkers	Category	Biomarkers concentrations Regarding to Eosinophil count		Biomarkers concentrations Regarding to Neutrophil count	
		No.	Mean ±SD	No.	Mean ±SD
SAA1 (unit)	High	65	12.3±7.65	32	14.9±8.8*
	Low	14	11.1±8.6	34	10.3±6.10
	Normal	21	16.1±10.1	34	13.7±9.68
	Total	100	12.94±8.3	100	11.3±14.7
	P value	0.136 NS		0.0064*	
IL-17 (unit)	High	65	221.5±112.9	32	200.51±288.9*
	Low	14	184.1±108.1	34	153.85±218.2
	Normal	21	255.2±133.6	34	195.3±285.6
	Total	100	223.3±117.5	100	200.1±246.7
	P value	0.212 NS		0.0072*	
IL-5 (unit)	High	65	262.8±194.8*	32	167.40±412.1
	Low	14	142.1±45.4	34	124.63±536.9
	Normal	21	196.1±3794	34	89.64±302.7
	Total	100	203.19±149	100	187.51±354.9
	P value	0.0015*		0.416 NS	
Periostin (unit)	High	65	95.5±49.5*	32	75.63±108.4
	Low	14	53.4±35.6	34	67.55±99.7
	Normal	21	82.2±40.1	34	55.47±79.4
	Total	100	80.1±431	100	72.42±89.5
	P value	0.0054*		0.740 NS	

*Mean significant difference at the 0.05 $p \leq 0.05$ by One way – ANOVA NS: Non-significant

3.3.2. Biomarkers according to mix granulocytic counts and FeNo levels:

The table (3-4) showed the concentrations of studied markers (SAA1, IL-17, IL-5, and Periostin) according to mix granulocytic and FeNo levels in asthmatic patients, which divided into two groups: Mix and normal for granulocytic counts, and high and normal for FeNo levels. The Statistical analysis using one way-ANOVA test showed highly-significant (P-value =0.009, P-value =0.0074, P-value =0.0021 and P-value =0.0013 respectively) for SAA1, IL-17, IL-5 and priostin, regarding to Mix granulocytic count, while both SAA1 and IL-17 effected significantly (P-value =0.0072 and P-value =0.0051 respectively) by FeNO levels. In the other hand, other biomarkers showed non-significant (p>0.05) differences.

Table (3-4): Biomarkers concentrations according to mix granulocytic count and FeNo levels:

Biomarkers	Biomarkers concentrations according to mix granulocytic count			Biomarkers concentrations According to FeNo Levels		
	Category	No.	Mean ±SD	Category	No.	Mean ±SD
SAA1 (unit)	Mix	22	17.021±9.5*	High	13	13.5±8.8*
	Normal	78	11.8±7.7	Normal	87	9.03±3.5
	Total	100	12.9±8.3	Total	100	12.9±8.3
	P value	0.009*			*0.0072**	
IL-17 (unit)	Mix	22	274.1±134.4*	High	13	232.2±120.5*
	Normal	78	209.1±109.1	Normal	87	164.1±73.3
	Total	100	223.4±117.5	Total	100	223.3±117.5
	P value	0.0074*			0.0051*	
IL-5 (unit)	Mix	22	412.9±600.1*	High	13	144.2±90.7
	Normal	78	231.2±350.9	Normal	87	290.1±448.1
	Total	100	271.1±421.7	Total	100	271.1±421.7
	P value	0.0021*			0.246^{NS}	
Periostin (unit)	Mix	22	101.1±519*	High	13	68.2±26.9
	Normal	78	75.3±38.8	Normal	87	82.8±44.7
	Total	100	80.1±43.1	Total	100	80.9±43.1
	P value	0.0013*			0.256 ^{NS}	
*Mean significant difference at the 0.05 p ≤ 0.05 by One way – ANOVA, NS: Non-significant						

3.3.3. Biomarkers according to Passive smoking:

The table (3-5) showed the distribution of biomarkers according to passive smoking. It is worth noting that the results of the statistical analysis did not document any significant differences in the distribution of these studied biomarkers according to passive smoking.

Table (3-5): Biomarker concentration according to Passive smoking

Biomarker / Passive smoking		No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
SAA1 (unit)	Passive smoking	48	13.589	6.591	0.132^{NS}
	Non-Passive smoking	52	11.405	5.714	
	Total	100	12.541	6.231	
IL-5 (unit)	Passive smoking	48	183.911	17.423	0.265^{NS}
	Non-Passive smoking	52	156.488	17.124	
	Total	100	170.748	12.249	
IL-17 (unit)	Passive smoking	48	259.704	20.109	0.138^{NS}
	Non-Passive smoking	52	219.442	17.662	
	Total	100	240.378	13.546	
Periostin (unit)	Passive smoking	48	83.195	40.683	0.591^{NS}
	Non-Passive smoking	52	78.535	32.622	
	Total	100	80.958	40.023	
NS: Non-Significant difference under $p \leq 0.05$ by T-test					

3.4. Frequencies of allergens in asthmatics patients:

The table (3-6), showed the results of the frequencies of all allergens in asthmatics patients. The highest frequency of those allergens among asthmatics patients in the current study were Cat (18%), cultivated oat (17%) Meadow foxtail (19%), Goosefoot (15%), Russian thistle (22%), Rough pigweed (16%), Cockroach Germany (9%) and Altrenaria Altrenaria (12%).

Table (3-6): Frequency of allergens in asthmatics patients

Allergens	Count (%)		Total
Cat	Positive	18%	100
	Negative	8 %	
Cultivated oat	Positive	17%	100
	Negative	83%	
Meadow foxtail	Positive	19%	100
	Negative	81%	
Goosefoot	Positive	15%	100
	Negative	85%	
Russian thistle	Positive	22%	100
	Negative	78%	
Rough pigweed	Positive	16%	100
	Negative	84%	
Cockroach Germany	Positive	9%	100
	Negative	91%	
Altrenaria Altrenaria	Positive	12%	100
	Negative	88%	

3.5. Impact of allergens on Eosinophil and neutrophil count:

The table (3-7) the effect of the presence (positive) or absence (negative) of the studied allergens (S.IgE) inhalation on eosinophil and neutrophil counts in asthmatic patients. The results of the statistical analysis showed only Rough pigweed allergen have highly significant differences (P-value =0.007) between eosinophil counts, which are distributed into high, low and normal. While the (Cultivated oat, Meadow foxtail, Cockroach Germany and Altrenaria Altrenaria) are have been show significant differences (P-value =0.005, P-value =0.005, P-value =0.002 and P-value =0.003 respectively) between neutrophilic counts, which are distributed into high, low and normal, whilst the other allergens did not document any significant differences in the distribution of these studied allergen.

Table (3-7) Impact of allergens in eosinophil and neutrophil counts

Allergens		Neutrophil count no. (%)				Eosinophil count no. (%)			
		High	Low	Normal	Total	High	Low	Normal	Total
Cat	Positive	6(33.3%)	8(44.4%)	4(22.2%)	18	12(66.7%)	2(11.1%)	4(22.2%)	18
	Negative	26(31.7%)	26(31.7%)	30(36.6%)	82	53(64.7%)	12(14.6%)	17(20.7%)	82
	Total	32	34	34	100	65	14	21	100
	P value	0.921 NS				0.447 NS			
Cultivated oat	Positive	3(17.6%)	10(58.8%)	4(23.5%)	17	12(70.6%)*	1(5.9%)	4(23.5%)	17
	Negative	29(34.9%)	24(28.9%)	30(36.1%)	83	53(63.9%)	13(15.7%)	17(20.2%)	83
	Total	32	34	34	100	65	14	21	100
	P value	0.570 NS				0.005*			
Meadow foxtail	Positive	4(21.1%)	11(57.9%)	4(21.1%)	19	12(63.2%)*	2(10.5%)	5(26.3%)	19
	Negative	28(34.6%)	23(28.4%)	30(37.0%)	81	53(65.4%)	12(14.8%)	16(19.8%)	81
	Total	32	34	34	100	65	14	21	100
	P value	0.765 NS				0.005*			
Goosefoot	Positive	4(26.7%)	7(46.7%)	4(26.7%)	15	12(80.0%)	2(13.3%)	1(6.7%)	15
	Negative	28(32.9%)	27(31.8%)	30(35.3%)	85	53(62.4%)	12(14.1%)	20(23.5%)	85
	Total	32	34	34	100	65	14	21	100
	P value	0.311 NS				0.530 NS			
Russian thistle	Positive	7(31.8%)	10(45.5%)	5(22.7%)	22	16(72.7%)	3(13.6%)	3(13.6%)	22
	Negative	25(32.1%)	24(30.8%)	29(37.2%)	78	49(62.8%)	11(14.1%)	18(23.1%)	78
	Total	32	34	34	100	65	14	21	100
	P value	0.612 NS				0.343 NS			
Rough pigweed	Positive	4(25.0%)*	8(50.0%)	4(25.0%)	16	13(81.3%)	3(18.8%)	0(0.0%)	16
	Negative	28(33.3%)	26(31.0%)	30(35.7%)	84	52(61.9%)	11(13.1%)	21(25.0%)	84
	Total	32	34	34	100	65	14	21	100
	P value	0.007*				0.336 NS			
Cockroach Germany	Positive	6(66.7%)	3(33.3%)	0(.0%)	9	8(88.9%)	0(.0%)	1(11.1%)	9
	Negative	26(28.6%)	31(34.1%)	34(37.4%)	81	57(62.6%)	14(15.4%)	20(22.0%)	81
	Total	32	34	34	100	65	14	21	100
	P value	0.257 NS				0.002*			
Altrenaria Altrenaria	Positive	2(16.7%)	8(66.7%)	2(16.7%)	12	10(83.3%)	0(.0%)	2(16.7%)	12
	Negative	30(34.1%)	26(29.5%)	32(36.4%)	88	55(62.5%)	14(15.9%)	19(21.6%)	88
	Total	32	34	34	100	65	14	21	100
	P value	0.255 NS				0.003*			

*Significant difference at the 0.05 level by chi-square test, NS: Non-Significant

3.6. Impact of allergens in biomarkers concentrations of asthmatics patients:

The table (3-8) showed the revealed the effects of allergens (Sweet vernal mix, Firebush, Sorrel, White ash, Tree Mix 4 and CCD markers), in the biomarkers concentrations, The results of the statistical analysis of concentrations of studied biomarkers SAA1, IL-17, IL-5 and priostin, displayed increase in concentration of IL-5 (Mean ±SD = 597.6±861.4), (p-value= 0.002) due to the effect of (Sweet vernal mix) allergen. And increase in concentration of SAA1 and IL-17 (Mean ±SD = 13.4±8.5 and 231.5±118.7 respectively), (p-value= 0.006 and p-value=0.003 respectively) due to the effect of (Firebush) allergen. And increase in concentration of IL-5 (Mean ±SD =684.5±1026.1), (p-value= 0.004) due to the effect of (Sorrel) allergen, which similar to the effect of Tree Mix4 (Mean ±SD =673.4±1168.9), (p-value= 0.005). And increase in concentration of IL-5 and priostin (Mean ±SD = 688.4±936.2 and 117.5±48.1 respectively), (p-value= 0.006 and p-value= 0.001 respectively) due to the effect of (White ash) allergen. And increase in concentration of IL-5 (Mean ±SD = 597.6±861.4), (p-value= 0.002) due to the effect of (Sweet vernal mix) allergen. At the end, increase in concentration of SAA1, IL-17 and priostin (Mean ±SD = 21.3±8.5, 335.1±90.9 and 259.3±408 respectively), (p-value= 0.004, p-value= 0.005 and p-value= 0.009 respectively) due to the effect of (CCD markers) allergen.

Table (3-8) Impact of allergens in biomarkers concentrations in asthmatics patients

Biomarkers / Allergens			Biomarker concentration In asthmatic patients			
			SAA1	**IL-17**	**priostin**	**IL-5**
		Count	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Sweet vernal mix	Positive	13	12.5±8.1	219.7±115.7	79.6±42.1	597.6±861.4
	Negative	78	15.2±10.5	247.9±131.7	89.5±49.9	222.4±287.5
	Total	100	0.280 NS	0.421 NS	0.445 NS	0.002*
P-value						
Firebush	Positive	10	13.4±8.5	231.5±118.7	64.8±32.2	111.8±49.1
	Negative	90	8.3±4.2	150.1±76.6	82.7±43.8	288.8±440.9
	Total	100	0.006*	0.003*	0.210 NS	0.215 NS
P-value						
Sorrel	Positive	4	12.7±8.4	220.5±116.6	80.4±43.1	684.5±1026.1
	Negative	96	17.1±7.5	290.6±137.1	91.96425	253.97000
	Total	100	0.317 NS	0.244 NS	0.004*	0.604 NS
P-value						
White ash	Positive	7	12.5±7.9	220.1±115.7	117.5±48.1*	688.4±936.2*
	Negative	93	17.7±12.6	267.4±141.1	78.2±41.6	239.7±346.3
	Total	100	0.117 NS	0.306 NS	0.006*	0.001*
P-value						
Tree Mix-4	Positive	4	12.7±8.1	222.2±117.5	79.7±42.4	673.4±1168.9*
	Negative	96	17.2±13.6	250.4±131.1	109.9±52.4	254.4±367.5
	Total	100	0.297 NS	0.640 NS	0.171 NS	0.005*
P-value						
CCD Markers	Positive	4	21.3±8.5*	335.1±90.9*	116.646.2	259.3±408*
	Negative	96	12.5±8.2	218.7±116.5	79.4±42.4	556.1±692.1
	Total	100	0.004*	0.005*	0.169 NS	0.009*
P-value						
* Significant difference under p ≤ 0.05 by One way – ANOVA						

3.7. Correlation analysis

The table (3-9) showed the Correlation analysis of studied biomarkers using Pearson's correlation coefficients, Using the correlation and regression analysis IL-5 level showed a significant (P<0.05) positive correlation with IL-17, SAA1, and Periostin . IL-17 has a significant (P<0.05) positive correlation with SAA1, and Periostin. SAA1 showed a significant (P<0.05) positive correlation with Periostin.

Table (3-9): Correlation among studied biomarkers in asthma patients

Correlations					
Biomarkers		**IL-5**	**IL-17**	**SAA1**	**Periostin**
IL-5	Pr. Correlation	1	0.407**	0.547**	0.494**
	Sig. (2-tailed)		0.000	0.000	0.000
	N	100	100	100	100
IL-17	Pr. Correlation		1	0.799**	0.750**
	Sig. (2-tailed)			0.000	0.000
	N			100	100
SAA1	Pr. Correlation			1	0.774**
	Sig. (2-tailed)				0.000
	N				100
Periostin	Pr. Correlation				1
	Sig. (2-tailed)				
	N				
** Correlation is significant at the 0.01 level (2-tailed).					

3.8. Receiver Operative Characteristic Curve Analysis:

3.8.1. ROC Curve Analysis for biomarker analyzed in patients with Specific IgE inhalation:

The Table (3-10) showed the Receiver Operative Characteristic Curve (ROC) analysis, which yielded a cut off value of SAA1, IL-17, IL-5 and Periostin (12.7375, 190.837 168.474 and 42.2575, respectively) for prediction of asthma disease in patients were positive for S. IgE. The overall AUC, sensitivity, and specificity for SAA1, IL-5, IL-17, and Periostin were as follows: (0.584, 0.569, 0.536, and 0.574), (0.406, 0.406, 0.516, and 0.906), and (0.778, 0.75, 0.694, and 0.306), respectively. Figure (3-1) illustrates the sensitivity and specificity values for SAA1, IL-17, IL-5, and Periostin in patients positive for S. IgE.

Table (3-10): ROC analysis of biomarkers in patients according to S. IgE

Biomarker	AUC	Sensitivity	Specificity	Cut-off	P-value
SAA1	0.584	0.406	0.778	12.7375	0.164
IL-17	0.536	0.516	0.694	190.837	0.549
IL-5	0.569	0.406	0.75	168.474	0.251
Periostin	0.574	0.906	0.306	42.2575	0.219

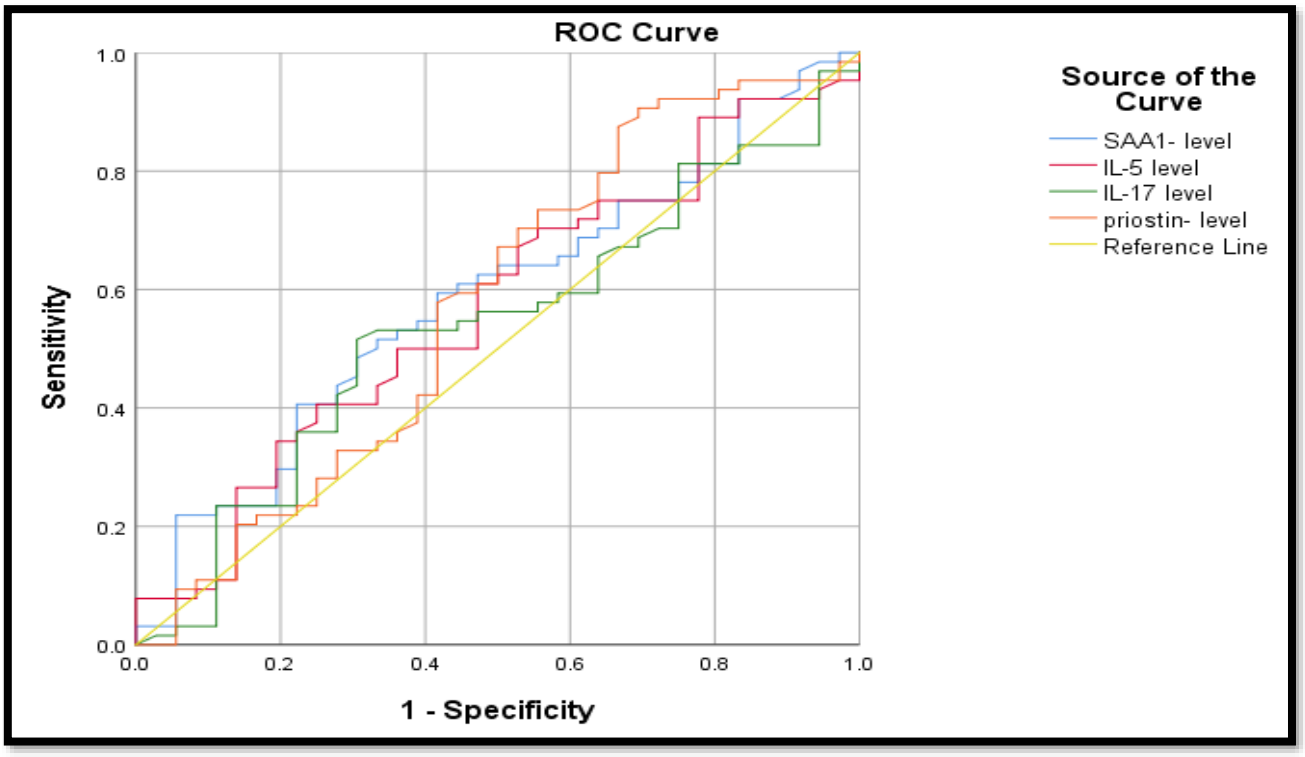


Figure (3-1): Roc curve for biomarkers analyzed in patients according to S. IgE

Chapter Four

Discussion

4. Discussion

4.1. Demographic date and patients' classification

This study underscores the importance of considering demographic factors in understanding health outcomes. The significant relationships observed can inform public health policies and interventions tailored to specific groups, particularly urban children and those with a family history of conditions.

The current study analysis reveals a significant difference (P = 0.0001) in age distribution suggesting that children aged 9–12 years constitute the majority (59%). This finding aligns with the study Banta *et al.*, (2021) conducted in California about asthma prevalence, this study highlighted that middle childhood is often a critical period for developing health-related conditions due to environmental and social exposures.

Regarding gender, the predominance of males (75%, P = 0.0001) aligns with observations in several pediatric studies that certain conditions, such as asthma or allergy-related illnesses, show higher prevalence among males in early life due to hormonal or immunological differences according to (Kim, Vazquez and Cubbin, 2023)

A significant urban predominance (65%, P = 0.0027) indicates better access to healthcare facilities or increased environmental triggers in urban areas. Urban settings often expose children to higher pollution levels, which can exacerbate conditions like asthma (Banta *et al.*, 2021). Regarding resident (urban vs. rural). However, literature generally supports that passive smoking is a risk factor for respiratory and allergic conditions in children(Kim, Vazquez and Cubbin, 2023). This discrepancy might be due to sample characteristics or confounding factors in the study.

The current study showed there was a strong association (P = 0.0001) between family history and asthma that suggests a genetic predisposition or shared

environmental factors within families. Studies consistently highlight the role of family history in conditions such as allergies and asthma (Kim, Vazquez and Cubbin, 2023).

Food allergy, there was a significant association (P = 0.0001) with food allergies (18%) reflecting global trends indicating increasing awareness and prevalence of such allergies. This emphasizes the need for targeted dietary interventions and early diagnosis (Banta *et al.*, 2021).

No significant association was observed for allergic rhinitis (P = 0.1615) or pet exposure (P = 0.6892). While these factors are documented as significant in other studies, variations might occur due to sample size, geographic differences, or the type of pets involved (Kim, Vazquez and Cubbin, 2023).

Regarding to the mixed granulocytic group, about 30% of patients have been elevated in count of both eosinophil and neutrophil count (mixed granulocytic). This align with (Shi *et al.*, 2021), with low observance of count percentage about 5% .

4.2. Classification of asthma patients according studied parameters:

The table (3-2) presents data on various immunological parameters and allergic markers, revealing statistically significant differences across categories for most parameters. The findings on lymphocyte, neutrophil, and eosinophil counts, as well as immunological markers, FeNO, IgE levels, provide valuable insights into allergic and inflammatory processes.

The lymphocyte distribution showed a significant predominance of normal levels (78%), with fewer individuals exhibiting high (15%) or low (7%) results. This aligns with the findings of Siroux *et al.*, (2024) highlighted that lymphocytes generally maintain homeostasis unless influenced by severe inflammation or systemic immunomodulation, while disagreed with Baraldo *et al.*, (2007) found chronic inflammation (asthma and COPD) were characterized by an increased number of types of lymphocytes and macrophages in the lung tissue and

neutrophils in the airway lumen. Lymphocytes, which are markedly different in the two inflammatory conditions, play a crucial role in the pathogenesis of asthma and COPD.

The neutrophil counts were balanced among groups, and showed high levels of neutrophil in (32%) of patients, low levels of neutrophil in (34%) of patients, and normal levels of neutrophil in (34%) of patients. In comparison with the study of Gungen and Aydemir, (2017) found elevated levels of neutrophils in the asthma group compared to the control, this interpretation is that cytokines in the pathogenesis of asthma cause an increase in neutrophils.

However, eosinophil levels were notably high in 65% of patients, consistent with their role in allergic and eosinophilic diseases such as asthma and allergic rhinitis. This is in line with studies of Tran *et al.*, (2014) and Bakakos, Loukides and Bakakos, (2019) found high blood eosinophil count associated with asthma, this suggests that eosinophil activation correlates with elevated IgE and cytokines, amplifying allergic responses.

The allergic level showed significant differences, with non-allergic patients being predominant (78%). This results lower than reported by Pakkasela *et al.*, (2020) who found the prevalence of 445 responders with physician-diagnosed asthma, 52% were classified as allergic and 48% as non-allergic. However, among those with allergic reactions, the mixed granulocytic level was elevated in 30% of individuals, supporting the notion of heterogeneous inflammatory responses in chronic allergic diseases (Breiteneder *et al.*, 2020).

FeNO levels were normal in 83% of patients, with only 17% showing high levels. High FeNO is a biomarker of inflammation and is commonly elevated in uncontrolled asthma and allergic airway disease. This result supported by Loewenthal and Menzies-Gow, (2022) who suggested Nitric oxide (NO) is exhaled in human breath and is a marker of airway inflammation. NO levels are increased in the exhaled breath of patients with type 2 asthma and fractional

exhaled nitric oxide (FeNO) provides an objective biomarker of airway inflammation. Conversely, total IgE was elevated in 32% of cases,

This in algin with Sherenian, Wang and Fulkerson, (2015) found the total serum IgE level was significantly elevated in children during asthma exacerbations requiring hospitalization compared to those seen in outpatient clinics. Elevated IgE levels are a hallmark of atopic conditions and have been linked to the activation of eosinophils via FcεRI receptors.

The specific IgE positivity was observed in 54% of patients, though it did not reach statistical significance (p=0.424). This discrepancy highlights the variability of specific IgE responses to allergens and emphasizes the need for a multifaceted approach to diagnosing and managing allergic conditions.

4.2.1. According to Eosinophil and Neutrophil groups (counts):

In this study, SAA1 levels did not differ significantly among patients with high, low, and normal eosinophil levels (p = 0.136). elevated SAA1 levels are linked to eosinophilic granulomatosis with polyangiitis (EGPA), where a positive correlation between SAA1 concentration and blood eosinophil counts was identified (Abouelasrar Salama *et al.*, 2023). This aligns with our findings that SAA1 concentration varies with eosinophil levels, though statistical significance was not achieved in our study. This suggests a need for further exploration of SAA1 as an inflammation biomarker in eosinophilic conditions.

Similarly, no significant difference was observed in IL-17 levels across eosinophil subgroups (p = 0.212), this inconsistent with (Ricciardolo *et al.*, 2017) found IL-17 significantly increased in asthmatic group. Regarding IL-5 levels showed a statistically significant difference among the groups (p = 0.0015), with the highest levels in patients with high eosinophils, this in line with Nagase, Ueki and Fujieda, (2020) reported IL-5 significantly increased with eosinophils in the asthmatic group, a role for IL-5 in eosinophil extracellular trap cell death, i.e., a proinflammatory cell death. IL-5 is widely recognized for its role in eosinophil

proliferation and activation in allergic diseases such as asthma (Bagnasco *et al.*, 2017). Periostin also displayed significant differences (p = 0.0054), being higher in patients with elevated eosinophil counts.

This consistency with Matsumoto, (2019) found serum periostin can serve as a valuable marker to identify patients with type-2 severe asthma as well as eosinophilic asthma, also show elevated serum periostin. Serum periostin may significantly help to improve the management of patients with severe asthma.

Regarding to the neutrophil counts in this study, SAA1 concentrations varied significantly among neutrophil levels (p = 0.0064). Patients with high neutrophil counts had the highest mean SAA1 concentration (14.88 ± 8.77), followed by the normal group (13.69 ± 9.54), and the lowest concentration in the low-neutrophil group (10.30 ± 6.04). These findings align with previous research Bich *et al.*, (2022) highlighting These results suggest that SAA1 levels are increased in neutrophilic airway inflammation and they may promote Poly I-C-induced inflammation in AECs through activating ERK/p38MAPK signalling pathway. Elevated SAA1 levels correlate with airway remodeling and disease severity in neutrophilic phenotypes of asthma.

IL-17 also showed significant variation across neutrophil counts (p = 0.0072). The high-neutrophil group demonstrated the highest mean IL-17 concentration (200.52 ± 288.89), followed by the normal group (195.29 ± 285.55), with the lowest levels in the low-neutrophil group (153.85 ± 218.18). This cytokine plays a pivotal role in promoting neutrophilic inflammation and has been implicated in steroid-resistant asthma. Elevated IL-17 levels in the high-neutrophil group reinforce its role in this asthma phenotype, supporting findings from studies that associate IL-17 with severe airway inflammation (Kang and Song, 2022).

No statistically significant differences were observed for IL-5 (p = 0.416) or periostin (p = 0.740) across neutrophil counts. IL-5, a key cytokine in eosinophilic inflammation, showed a mean concentration of 167.41 ± 413.00 in the high-neutrophil group, decreasing to 89.65 ± 302.65 in the normal group. Periostin levels ranged from 75.63 ± 108.34 in the high-neutrophil group to 55.48 ± 79.42 in the normal group. This align with Kang and Song, (2022) that suggest the lack of significance may indicate that these markers are more relevant to eosinophilic rather than neutrophilic asthma.

This study underscores the role of SAA1 and IL-17 as biomarkers for neutrophilic asthma, reflecting the distinct inflammatory pathways involved. Their significant association with neutrophil levels highlights their potential utility in identifying and managing non-eosinophilic asthma phenotypes. Conversely, the non-significant variation in IL-5 and periostin emphasizes the heterogeneity of asthma and the need to tailor biomarker panels to specific phenotypes. The findings support personalized medicine approaches, where biomarkers like SAA1 and IL-17 guide targeted therapies, potentially improving outcomes in patients with neutrophilic asthma.

4.2.2. Biomarkers according to mixed granulocytic counts and FeNo levels:

This study highlights significant differences in biomarker concentrations between mixed granulocytic and non-mixed granulocytic asthma phenotypes, providing insights into the inflammatory profiles of these groups. The mean SAA1 concentration in mixed granulocytic patients was significantly higher (17.02 ± 9.52 units) compared to non-mixed patients (11.79 ± 7.71 units, p = 0.009). this result agreed with Bich *et al.*, (2022) reported that the highest levels of SAA1 and neutrophilia were noted in the BALF and sera of the non-mixed granulocytic (NA) mouse model, followed by the mixed granulocytic asthma (MA) model.

Elevated SAA1 levels in mixed granulocytic asthma could indicate a pronounced inflammatory state, consistent with its role as an acute-phase protein linked to airway remodelling and systemic inflammation. Patients with the mixed granulocytic phenotype exhibited significantly higher IL-17 levels (274.02 ± 134.43 units) compared to non-mixed cases (209.09 ± 109.05 units, $p = 0.0074$). IL-17 has been strongly associated with neutrophilic inflammation and airway remodeling in asthma, particularly in steroid-resistant forms. The study of Al-Harbi *et al.*, (2019) had demonstrated that IL-17 in mice promotes the recruitment of neutrophils and exacerbates airway inflammation, as well as the same study showed that sulforaphane administration reduced neutrophilic airway inflammation and Th17 immune responses in a mixed granulocyte mouse model of asthma. On the other hand, corticosteroid treatment decreased Th2/eosinophilic immune responses but had little on Th17/neutrophilic immune responses.

The IL-5 concentration was notably elevated in mixed granulocytic asthma (412.86 ± 600.17 units) compared to non-mixed phenotypes (231.23 ± 350.85 units, $p = 0.0021$). IL-5 is a hallmark of eosinophilic inflammation, yet its elevated presence in mixed phenotypes may reflect the overlapping inflammatory processes characteristic of these patients. This supports findings of Chu *et al.*, (2015) that found inhibition of neutrophilic and eosinophilic inflammation was associated with markedly lower activating and chemotactic cytokines in the lung, IL-17A and and IL-5.

Mean periostin levels were significantly higher in the mixed granulocytic group (101.00 ± 51.81 units) compared to non-mixed cases (75.30 ± 38.73 units, $p = 0.0013$). Periostin, an extracellular matrix protein, is implicated in airway remodeling and both eosinophilic and neutrophilic inflammation. Its elevated levels in mixed phenotypes reinforce its potential as a marker for identifying complex inflammatory profiles in asthma. This findings inconsistency with result of Bobolea *et al.*, (2015) found periostin levels were higher in patients with fixed as compared to variable airflow limitation (69.76 *vs.* 43.84 pg/ml, $P < 0.05$) and

in patients with eosinophilic as compared to mixed granulocytic phenotype (61.58 *vs.* 37.31 pg/ml, $P < 0.05$).

Regarding to FeNo levels in this study, evaluates fractional exhaled nitric oxide (FeNO) levels in asthma patients and their association with biomarkers of inflammation. Elevated FeNO levels, indicative of airway inflammation, are compared to normal FeNO levels to explore variations in key biomarkers. Patients with high FeNO levels exhibited significantly elevated SAA1 concentrations (13.52 ± 8.74 units) compared to those with normal FeNO (9.03 ± 3.52 units, $p = 0.0072$). SAA1 is known to contribute to airway inflammation through the recruitment of inflammatory cells and promotion of cytokine release. Elevated levels in the high FeNO group suggest an association with severe or uncontrolled asthma.

IL-17 levels were significantly higher in the high FeNO group (232.23 ± 120.59 units) than normal FeNO (164.12 ± 73.32 units, $p = 0.0051$). IL-17, a cytokine associated with neutrophilic inflammation, may contribute to steroid resistance and airway hyperresponsiveness in high FeNO asthma. Researches of Chien *et al.*, (2013) and Badar *et al.*, (2020) It was highlighted that Serum IL-17 and FeNO levels were significantly higher in asthma patients and that IL-17 plays a role in exacerbating airway inflammation through neutrophil recruitment and epithelial cell activation.

IL-5 concentrations were higher in the high FeNO group (290.17 ± 448.11 units) compared to the normal FeNO group (144.22 ± 90.76 units), this difference was not statistically significant ($p = 0.246$). IL-5 is a key driver of eosinophilic inflammation, yet its variability suggests that FeNO levels may not always correlate with eosinophilic biomarkers. This is consistent with study of Howell *et al.*, (2024) showing that FeNO-high participants had over 2-fold higher sputum IL-5 concentrations.

Periostin levels were slightly elevated in the high FeNO group (82.86 ± 44.74 units) compared to normal FeNO (68.26 ± 26.95 units), but this difference was not significant ($p = 0.256$).

Periostin plays a role in airway remodeling and Th2-driven inflammation, aligning with its moderate elevation in high FeNO cases. The lack of statistical significance may reflect the complex interplay of FeNO with Th2 and non-Th2 pathway, while a study of Nagasaki, Matsumoto and Izuhara, (2017) showed the level of Periostin increased significantly with FeNO in asthmatic pateints.

4.2.3. Biomarkers according passive smoking

This study evaluated the impact of passive smoking on inflammatory biomarkers in asthma patients. The results indicate slight variations in the concentrations of SAA1, IL-5, IL-17, and periostin between passive smokers and non-passive smokers, The mean SAA1 concentration was slightly higher in passive smokers (13.59 ± 6.59 units) compared to non-passive smokers (11.41 ± 5.71 units), with $p = 0.132$. While not significant, the elevated levels align with findings suggesting that passive smoking exacerbates airway inflammation by promoting systemic inflammatory markers such as SAA1 (Strzelak *et al.*, 2018). This indicates that passive smoke exposure may contribute to a heightened inflammatory state, potentially worsening asthma severity.

Passive smokers demonstrated marginally higher IL-5 concentrations (183.91 ± 17.42 units) compared to non-passive smokers (156.49 ± 17.12 units), with $p = 0.265$. IL-5, a cytokine crucial for eosinophilic inflammation, has been implicated in asthma pathophysiology. Although passive smoking has been shown to elevate eosinophilic activity (Calogero *et al.*, 2019). This study's lack of significance suggests that passive smoke exposure may only partially modulate IL-5 levels.

IL-17 levels were also higher among passive smokers (259.70 ± 20.11 units) compared to non-passive smokers (219.44 ± 17.66 units), with $p = 0.138$. IL-17 plays a role in neutrophilic inflammation and airway remodeling, which may be exacerbated by passive smoke exposure.

The observed trend aligns with research of Javed *et al.*, (2020) highlighting the association between tobacco smoke and IL-17-driven inflammation in asthma, that explained that salivary IL-17A levels were higher in marijuana-smokers than cigarette-smokers ($p < 0.01$) and non-smokers-with-periodontitis ($p < 0.01$). Whole salivary IL-17 levels were higher among cigarette-smokers than non-smokers with periodontitis ($p < 0.01$) and periodontally-healthy-individuals ($p < 0.01$). Marijuana- and heavy cigarette-smokers have comparable clinic-radiographic periodontal statuses.

The periostin levels showed no significant difference between passive smokers (83.20 ± 40.68 units) and non-passive smokers (78.54 ± 32.62 units), with $p = 0.591$. Periostin, a marker of Th2 inflammation and airway remodeling, may not be strongly influenced by passive smoking. This is consistent with prior findings El Basha *et al.*, (2018) that non-significant relation was found between passive smoking and periostin levels in all asthmatic group. This findings suggest that while passive smoking may elevate inflammatory biomarkers in asthma patients, its effects may not be as pronounced as those observed with active smoking.

4.3. Frequencies of allergens in asthmatics patients

The data highlights significant sensitization to several allergens among the studied group including cats (18%), cultivated oat (17%), meadow foxtail (19%), and Russian thistle (22%). These findings align with previous studies emphasizing the role of these allergens in exacerbating allergic conditions like asthma. Sensitization to allergens is a critical factor in asthma management and is commonly associated with heightened airway inflammation and symptom

severity. Sensitization to cat allergens, particularly *Fel d 1*, is a well-recognized trigger in asthma patients. A significant portion of sensitized individuals exhibits heightened reactivity, often requiring targeted allergen avoidance or immunotherapy (Satyaraj et al., 2019).

Grass pollens, including cultivated oat and meadow foxtail, are dominant allergens. Their impact is substantial due to widespread environmental exposure and cross-reactivity with other grass species (Taketomi *et al.*, 2006). The high sensitization rates observed (17–19%) may reflect regional pollen profiles and patient demographics.

Sensitization to weed pollens like Russian thistle (22%) and goosefoot (15%) is consistent with global patterns of allergen sensitization in semi-arid regions. These weeds release highly allergenic pollen, often linked to seasonal exacerbations of asthma (Sierra-Heredia *et al.*, 2018).

The 12% sensitization rate to *Alternaria alternata* is significant given its association with severe asthma phenotypes. This allergen is particularly concerning in humid conditions, where fungal growth is prevalent (Bush and Portnoy, 2001).

Approximately 16% of the subjects showed sensitization to rough pigweed (*Amaranthus retroflexus*). This aligns with studies identifying rough pigweed as a common trigger in regions with high weed pollen exposure, particularly in late summer and fall. Sensitization is often associated with increased IgE levels and clinical symptoms such as rhinitis and asthma exacerbations. Weed allergens like rough pigweed are highly prevalent in arid and semi-arid climates (Bassett Healthcare Network, 2023).

The prevalence of sensitization to German cockroach (*Blattella germanica*) was 9%. Cockroach allergens are well-documented indoor triggers for allergic reactions, particularly in urban environments. Exposure to these allergens is strongly linked to persistent asthma and allergic rhinitis. Previous research highlights that sensitization to cockroach allergens correlates with

increased asthma severity, The first report on positive skin test responses to cockroach allergen dates back to 1964 (Dramburg *et al.*, 2023).

A strong association between cockroach allergy, allergic rhinitis, and asthma has been demonstrated (Pomés, Chapman and Wünschmann, 2016). Inner-city asthma studies in the USA have shown that exposure and sensitization to cockroach allergens are associated with increased asthma morbidity in children (Gruchalla *et al.*, 2005). The two most common species are German and American cockroach (*Blattella germanica* and *Periplaneta americana*, respectively).

Sensitization to *Alternaria alternata* was found in 12% of participants. This fungal allergen is a critical trigger for respiratory allergic diseases, including asthma. Studies have shown that *Alternaria alternata* produces potent allergens capable of eliciting strong immune responses, leading to exacerbations in patients with atopic conditions (Giusti *et al.*, 2024). The global prevalence of sensitization to fungal allergens varies but remains a significant concern in damp and humid environments.

The high prevalence of sensitization underscores the importance of integrating allergen testing into asthma management strategies. Allergen-specific interventions, including immunotherapy and environmental controls, can mitigate symptoms and improve quality of life. For example, reducing exposure to cat allergens through targeted interventions has shown to significantly benefit patients with cat-induced asthma.

4.3.1. Impact of allergens on Eosinophil and neutrophil count:

This study evaluated the relationship between sensitization to common allergens and eosinophil counts (high, low, or normal) in allergic patients. Sensitization to most aeroallergens, including cat allergens (p=0.921), cultivated oat (p=0.570), meadow foxtail (p=0.765), goosefoot (p=0.311), Russian thistle (p=0.612), German cockroach (p=0.257), and *Alternaria alternata* (p=0.255), was not significantly associated with eosinophil counts. Despite the lack of statistical

significance, sensitization to these allergens was more frequently observed in patients with high eosinophil counts.

For instance, 66.7% of patients sensitized to cat allergens and 70.6% sensitized to cultivated oat had high eosinophil counts. This is consistent with previous findings linking elevated eosinophil counts to allergic sensitization and inflammation in atopic patients (Matucci *et al.*, 2018; Beck and Leung, 2000;). Sensitization to rough pigweed (*Amaranthus retroflexus*) showed a significant association with eosinophil counts (p=0.007).

Of those sensitized, 81.3% had high eosinophil counts, while none had normal counts. This finding highlights rough pigweed as a potentially important allergen in driving eosinophilic inflammation in sensitized individuals. This finding suggests rough pigweed as a potent allergen influencing eosinophilic inflammation. Supporting research has identified *Amaranthus retroflexus* pollen as a common trigger for allergic rhinitis and asthma in areas with substantial weed growth and environmental pollen exposure (Arbes Jr *et al.*, 2007; D'Amato *et al.*, 2010). Eosinophils are key markers of allergic inflammation, often elevated in patients with atopy, asthma, and allergic rhinitis. Sensitization to specific aeroallergens, as observed in this study, may exacerbate eosinophilic inflammation, thereby worsening clinical outcomes.

Regarding to the neutrophil counts, the study explores the association between exposure to various allergens and neutrophilic counts among asthma patients. Neutrophilic inflammation is often linked to non-allergic asthma phenotypes, but its relationship with specific allergens has been less explored.

The analysis of neutrophilic counts and their association with allergen sensitization in asthma patients highlights distinct patterns. For instance, cat allergens were associated with higher percentages of neutrophilic inflammation (33.3% high neutrophilic counts among positive cases). However, this was

comparable to negative cases (31.7%). Similar findings are observed with goosefoot (26.7% high counts for positive vs. 32.9% for negative cases).

These results suggest that sensitization to some allergens does not substantially alter neutrophilic inflammation, reflecting potentially complex interactions between allergen exposure and innate immune responses.Conversely, for cultivated oat and meadow foxtail, the proportion of individuals with high neutrophilic counts was lower in sensitized individuals (17.6% and 21.1%, respectively) compared to nonsensitized cases (34.9% and 34.6%). This may imply that sensitization to these allergens could be less associated with neutrophilic-driven asthma exacerbations.

For Rough pigweed, 25% of sensitized individuals had high neutrophilic counts, 50% had low counts, and 25% exhibited normal counts. The p-value (0.336, non-significant) suggests no strong association between Rough pigweed sensitization and neutrophilic inflammation. The Cockroach Germany allergen demonstrated a significant association with high neutrophilic counts (66.7% of sensitized individuals). The p-value of 0.002 underscores this relationship. For Altrenaria Altrenaria, 16.7% of sensitized individuals had high neutrophilic counts, and 66.7% showed low counts, with a statistically significant p-value of 0.003. Mold allergens like Alternaria are recognized for their ability to induce neutrophilic asthma.

The data align with findings by (Chung, 2018), who emphasized the heterogeneous inflammatory responses in asthma, including the overlapping presence of neutrophilic and eosinophilic inflammation, particularly in severe cases. Similarly, Fahy, Corry and Boushey, (2000) described that neutrophilic asthma often correlates with exposure to environmental pollutants rather than allergens, a factor that may explain the modest changes observed in the current study. Moreover, Russell and Brightling, (2017) noted that neutrophil recruitment

in the airways might result from mechanisms independent of classic allergen sensitization, including microbial colonization or infection.

This could explain why high neutrophilic counts are not consistently elevated across all allergen-positive groups. Study of Douwes *et al.*, (2002) suggesting that plant-based allergens are more commonly associated with eosinophilic rather than neutrophilic inflammation, Mold allergens like Alternaria are recognized for their ability to induce neutrophilic asthma, likely due to their proteolytic enzymes and activation of innate immune pathways (Bush and Prochnau, 2004).

While previous findings inconsistent with this study, Wenzel, (2012) argues that while neutrophilic inflammation is prominent in severe asthma, its association with specific allergens is inconsistent and often overshadowed by non-allergic triggers like infections or environmental factors. Haldar *et al.*, (2008) showed that allergen sensitization is more strongly linked with Th2-high inflammation (eosinophilic) rather than neutrophilic pathways, which are typically associated with non-allergic asthma exacerbations.

4.4. Impact of allergens in biomarkers concentrations of asthmatics patients:

The results indicate that biomarker concentrations in patients sensitized to the Sweet Vernal Mix aeroallergen show variable significance. Among the biomarkers analyzed, IL-5 demonstrated a statistically significant difference, while SAA1, IL-17, and periostin levels did not show significant variation between sensitized and non-sensitized individuals.

The mean SAA1 concentration was slightly lower in patients sensitized to Sweet Vernal Mix (12.59 ± 8.02 units) compared to non-sensitized individuals (15.29 ± 10.52 units), though this difference was not statistically significant ($p = 0.280$). These findings suggest that Sweet Vernal Mix exposure does not markedly influence systemic inflammation as measured by SAA1.

This is consistent with studies indicating that SAA1 levels are more reflective of acute-phase inflammatory responses than allergen-specific immune activation (Uhlar and Whitehead, 1999).

IL-17 concentrations were also not significantly different between sensitized (219.70 ± 115.65 units) and non-sensitized patients (247.97 ± 131.71 units) ($p = 0.421$). The absence of significant differences suggests that the immune response triggered by sensitization to Sweet Vernal Mix allergens does not strongly involve or activate the Th17 pathway, which is responsible for producing IL-17. This implies that this specific allergen might not substantially influence Th17-mediated inflammation in asthma, distinguishing it from other allergens that may play a more active role in exacerbating such pathways.

While IL-5 levels were significantly elevated in Sweet Vernal Mix-sensitized patients (597.63 ± 861.48 units) compared to non-sensitized individuals (222.42 ± 287.55 units) ($p = 0.002$). This result highlights the role of Sweet Vernal Mix in promoting a Th2-skewed immune response, with IL-5 being central to eosinophil recruitment and activation.

Periostin concentrations were slightly lower in Sweet Vernal Mix-sensitized patients (79.68 ± 42.07 units) compared to non-sensitized individuals (89.52 ± 49.92 units), but the difference was not significant ($p = 0.445$). Periostin is a biomarker of airway remodeling and Th2-driven inflammation, often associated with chronic asthma (James et al., 2020). The lack of significant differences suggests that Sweet Vernal Mix exposure does not induce substantial periostin-mediated structural changes in this patient group.

The analysis of biomarker concentrations in patients sensitized to Firebush (Positive) compared to non-sensitized individuals (Negative) reveals significant findings for certain biomarkers:

Sensitized patients showed significantly higher serum amyloid A1 (SAA1) levels (13.45 ± 8.58 units) compared to non-sensitized individuals (8.37 ± 4.22 units, $p = 0.006$). This elevation suggests that exposure to Firebush aeroallergens may provoke acute-phase inflammatory responses, as SAA1 is a known marker of systemic inflammation and immune activation. Previous study have shown that SAA1 levels are elevated in patients with allergic asthma, particularly during exacerbations triggered by environmental allergens (Choy *et al.*, 2015).

IL-17 concentrations were also significantly higher in sensitized patients (231.53 ± 118.74 units) compared to non-sensitized individuals (150.05 ± 76.67 units, $p = 0.003$). This suggests that Firebush sensitization activates the Th17 immune pathway, which is implicated in neutrophilic airway inflammation and steroid-resistant asthma (Choy *et al.*, 2015; Al-Ramli *et al.*, 2009). The elevated IL-17 levels may contribute to enhanced neutrophilic infiltration and airway remodeling.

IL-5 concentrations were not significantly different between sensitized (111.89 ± 49.08 units) and non-sensitized patients (288.90 ± 440.95 units, $p = 0.210$). IL-5 is central to eosinophilic inflammation, and its lack of significant variation suggests that Firebush sensitization does not predominantly drive eosinophil-mediated responses in this study. This aligns with studies indicating variability in IL-5 expression depending on allergen type and individual patient phenotypes (Fahy, 2015). Periostin concentrations also did not differ significantly between sensitized (64.88 ± 32.29 units) and non-sensitized patients (82.74 ± 43.83 units, $p = 0.215$). While periostin is a biomarker of type 2 inflammation, its non-significance here may point to a reduced role of epithelial remodeling in Firebush sensitization compared to other aeroallergens.

These findings emphasize the heterogeneity of immune responses to aeroallergens, with Firebush notably activating Th17-mediated pathways rather than traditional type 2 immune responses. This highlights the importance of

individualized approaches in managing allergic asthma, particularly in patients with specific sensitization profiles.

Regarding to the Sorrel sensitization, SAA1 Concentrations were not significantly different between sensitized and non-sensitized groups ($p = 0.317$). Serum amyloid A (SAA1) is a marker of systemic inflammation and acute-phase response, but its role in allergen-specific pathways, such as Sorrel sensitization, remains limited. IL-17 did not significantly differ ($p = 0.244$), suggesting that Th17-driven neutrophilic inflammation may not be strongly linked to Sorrel sensitization. Elevated IL-17 levels are known to correlate with severe asthma phenotypes but vary by allergen-specific activation mechanisms (Rahmawati *et al.*, 2021).

IL-5, a significant increase was noted in sensitized patients ($p = 0.004$). IL-5, central to eosinophilic inflammation, aligns with Th2-driven asthma pathology, indicating that Sorrel allergens likely activate Th2 pathways more strongly than Th17 (Rahmawati *et al.*, 2021). Periostin showed no significant differences were found ($p = 0.604$). As a biomarker of airway remodeling in Th2-high asthma, its lack of variation may reflect minimal remodeling differences between the groups.

Regarding to the White Ash sensitization, SAA1 showed no significant difference in concentrations ($p = 0.117$), indicating that systemic inflammation driven by White Ash sensitization may not differ significantly between sensitized and non-sensitized patients. As well as, IL-17 showed no significant variation ($p = 0.306$), suggesting limited involvement of Th17-mediated neutrophilic inflammation in response to White Ash. While IL-5 a significant increase in sensitized patients ($p = 0.006$) highlights Th2 pathway activation, characteristic of eosinophilic asthma responses to allergens and Periostin showed a significant increase in sensitized patients ($p = 0.001$) underscores its role in airway remodeling, often seen in Th2-high asthma phenotypes.

White ash (*Fraxinus excelsior*) is a known allergen, and exposure to its pollen can contribute to various allergic reactions, including respiratory symptoms like asthma. In particular, individuals with asthma may experience exacerbated symptoms, such as wheezing, coughing, and shortness of breath, when sensitized to white ash pollen. This is largely due to the airway inflammation and immune responses triggered by the allergen, which often involves a complex interaction between environmental factors and genetic predispositions (Niederberger *et al.*, 2002). The relationship between white ash (*Fraxinus excelsior*) and SAA1, IL-5, IL-17 and periostin in asthmatic patients is not extensively studied, but there is some indirect connection through the immune response in asthma. Which can rise in response to inflammation, infections, and allergic reactions, all of which are common in asthma exacerbations. They plays a role in the inflammatory response and is elevated in individuals with various respiratory conditions, including asthma many airborne allergens, can trigger an allergic response in sensitized individuals, leading to an inflammatory cascade.

Regarding to the White Ash sensitization, the analysis of biomarkers in asthma patients with and without sensitization to Tree Mix 4 revealed several key findings that highlight the varying impacts of allergen sensitization on inflammatory pathways.

The mean SAA1 levels in Tree Mix 4-sensitized individuals were not significantly different from those in non-sensitized individuals ($p=0.297$). SAA1 is an acute-phase reactant often associated with inflammation and asthma exacerbations. While elevated SAA1 has been linked to more severe asthma phenotypes and Th17 inflammation in prior studies, the lack of significant variation here might suggest that Tree Mix 4 sensitization does not strongly influence systemic inflammation mediated through SAA1 pathways (Woodruff *et al.*, 2009).

IL-17 levels were also not significantly different between sensitized and non-sensitized groups (p=0.640). IL-17 is a hallmark of Th17-mediated neutrophilic inflammation, often seen in more severe or corticosteroid-resistant asthma. The non-significant trend in IL-17 concentrations suggests that Tree Mix 4 sensitization may not robustly activate Th17-driven responses in the studied group. This aligns with findings from other studies indicating variability in IL-17 levels depending on the allergen type and asthma phenotype (Östling *et al.*, 2019).

IL-5 levels were significantly higher in Tree Mix 4-sensitized patients (p=0.005), indicating activation of Th2-mediated eosinophilic inflammation. IL-5 is central to eosinophil recruitment and activation, which contribute to airway inflammation and hyperresponsiveness in allergic asthma. The marked elevation of IL-5 in the sensitized group underscores the role of Tree Mix 4 in driving Th2 responses, consistent with studies linking allergen exposure to heightened eosinophilic activity (Ahlstrom-Emanuelsson *et al.*, 2004).

Although periostin levels were slightly higher in the sensitized group, the difference was not statistically significant (p=0.171). Periostin, an extracellular matrix protein, is often associated with airway remodeling and Th2 inflammation in asthma (Izuhara *et al.*, 2017). The non-significant result may reflect variability in periostin expression among patients or the influence of other factors not accounted for in this study.

Regarding to the CCD markers sensitization, the analysis of biomarkers in patients sensitized to cross-reactive carbohydrate determinant (CCD) markers highlights their potential role in modulating immune responses in asthma.

SAA1 concentrations were significantly higher in CCD-sensitized patients (mean: 21.31 ± 8.52) compared to non-sensitized patients (mean: 12.59 ± 8.23, p=0.004). SAA1 is an acute-phase protein linked to systemic inflammation and exacerbations in asthma.

Elevated levels in CCD-sensitized patients suggest a possible connection between CCD-related immune activation and heightened systemic inflammatory responses. No study for compared.

IL-17 levels were significantly elevated in CCD-sensitized patients (mean: 335.19 ± 90.97) compared to non-sensitized individuals (mean: 218.71 ± 116.55, p=0.005). IL-17 is pivotal in neutrophilic inflammation and steroid-resistant asthma phenotypes. The heightened IL-17 levels in CCD-sensitized individuals may indicate a stronger Th17-mediated inflammatory response, supporting previous reports linking IL-17 to exacerbated airway inflammation in allergen-sensitized asthma cohorts (McKinley *et al.*, 2008).

IL-5 levels did not show a significant difference between CCD-sensitized and non-sensitized groups (p=0.169). This lack of significant difference may indicate that CCD sensitization has a lesser impact on Th2-driven eosinophilic pathways compared to other allergens. IL-5's role in eosinophil recruitment and activation remains crucial in allergic asthma, but its involvement appears to vary with allergen type and immune response. No study found for comparable

Periostin levels were significantly elevated in CCD-sensitized patients (mean: 116.69 ± 46.29) compared to non-sensitized individuals (mean: 79.47 ± 42.49, p=0.009). Periostin is strongly associated with Th2 inflammation and airway remodeling in asthma. The elevated periostin levels in CCD-sensitized patients may reflect increased extracellular matrix deposition and airway remodeling, reinforcing its role as a biomarker for asthma severity and Th2-skewed inflammation (Izuhara *et al.*, 2017).

The significant elevations in SAA1, IL-17, and periostin levels in CCD-sensitized individuals highlight the diverse immunological pathways activated by CCD markers. These findings suggest that CCD sensitization contributes to both systemic and localized airway inflammation, potentially exacerbating asthma severity. However, the non-significant IL-5 results indicate that the role of Th2

responses in CCD sensitization may be less pronounced compared to Th17 pathways and systemic inflammation.

4.5. Correlation among Studied Biomarkers in Asthma Patients

The analysis reveals significant correlations among key biomarkers in asthma patients, suggesting interconnected pathways in the immunological and inflammatory processes underlying the disease.

IL-5 showed a significant positive correlation with IL-17 ($r=0.407$, $p<0.001$), SAA1 ($r=0.547$, $p<0.001$) and periostin ($r=0.494$, $p<0.001$). This indicates that IL-5, a cytokine pivotal for eosinophil activation and survival, is closely linked to both Th2 and Th17 inflammatory pathways, consistent with studies suggesting crosstalk between Th2 and Th17-mediated responses in asthma (Bullens *et al.*, 2006). The negative correlation between IL-5 and total IgE ($r=-0.239$, $p=0.017$) suggests a complex regulation of IgE production, possibly influenced by differential allergen sensitizations and Th2 dynamics (Holgate, 2012).

IL-17 exhibited strong positive correlations with SAA1 ($r=0.799$, $p<0.001$) and periostin ($r=0.750$, $p<0.001$). This highlights the role of IL-17 in neutrophilic inflammation and airway remodeling, reinforcing its association with biomarkers of inflammation and structural changes like periostin, this in line with study of Tang *et al.*,(2017) that found IL-17, Th17 highly and positively correlated with SAA1 expression

The negative correlation with total IgE ($r=-0.230$, $p=0.021$) may reflect IL-17's predominant role in non-IgE-mediated inflammatory pathways, which are often associated with steroid-resistant asthma phenotypes (Dunican and Fahy, 2015). SAA1 correlated significantly with periostin ($r=0.774$, $p<0.001$), reflecting its involvement in airway remodeling and inflammation. The lack of significant correlation with total IgE ($r=-0.158$, $p=0.117$) suggests that SAA1-driven inflammation operates independently of IgE-mediated pathways, likely reflecting its acute-phase protein characteristics

Periostin, a marker of Th2 inflammation and airway remodeling, negatively correlated with total IgE ($r=-0.299$, $p=0.002$), potentially indicating differential contributions of periostin in airway structural changes unrelated to IgE-mediated allergic responses (Wenzel, 2012).

4.5.1. ROC Analysis of Biomarkers in Patients According to S. IgE

The Receiver Operating Characteristic (ROC) analysis evaluates the diagnostic performance of biomarkers for differentiating asthma patients based on serum IgE (S. IgE) levels. Below is a detailed discussion of the results:

4.5.1.A. SAA1:

SAA1 (Serum Amyloid A1): AUC = 0.584: SAA1 demonstrated poor diagnostic accuracy, as an AUC value between 0.5 and 0.6 reflects minimal ability to distinguish between patient groups.

Sensitivity = 40.6% and Specificity = 77.8%: While specificity is relatively moderate, sensitivity is low, indicating limited reliability in detecting IgE-mediated asthma phenotypes.

Cut-off = 12.7375 units: Patients with SAA1 levels above this threshold may exhibit increased inflammatory responses; however, the non-significant $p=0.164$ suggests that SAA1 lacks strong diagnostic utility in this context.

Context in Asthma: Previous research highlights SAA1 as an acute-phase reactant associated with systemic inflammation. Its weak correlation with S. IgE suggests that it may be more relevant to non-IgE-mediated inflammatory pathways (Wenzel, 2012).

4.5.1.B. IL-17:

AUC = 0.536: IL-17 also showed poor discrimination for IgE levels, with an AUC slightly above 0.5.

Sensitivity = 51.6% and Specificity = 69.4%: These values reflect a marginal ability to identify IgE-mediated inflammation.

Cut-off = 190.837 units, p=0.549: The lack of statistical significance implies that IL-17 is not a reliable marker for differentiating IgE-related asthma phenotypes in this cohort.

Context in Asthma: IL-17 is more commonly associated with non-allergic, neutrophilic asthma rather than IgE-mediated responses. Its presence reflects Th17-driven inflammation, which may coexist with Th2-mediated processes in asthma patients (Bullens *et al.*, 2006; McKinley *et al.*, 2008)

4.5.1.C. IL-5:

AUC = 0.569: IL-5 exhibited poor discriminatory power in identifying IgE-mediated phenotypes.

Sensitivity = 40.6% and Specificity = 75%: Similar to SAA1, low sensitivity limits its diagnostic utility, despite a moderate specificity.

Cut-off = 168.474 units, p=0.251: The non-significant ppp-value underscores its limited role as a biomarker for IgE-dominant asthma.

Context in Asthma: IL-5 plays a central role in eosinophilic asthma, a phenotype that often overlaps with IgE-mediated mechanisms. However, its modest diagnostic performance in this study suggests variability in its predictive value for S. IgE (Fahy, 2015).

4.5.1.D. Periostin:

AUC = 0.574: Periostin had slightly better diagnostic accuracy than IL-17 and IL-5 but remains insufficient for robust differentiation.

Sensitivity = 90.6% and Specificity = 30.6%: High sensitivity indicates that periostin is effective at detecting patients with elevated IgE, but its low specificity limits its ability to exclude those without IgE-mediated inflammation.

Cut-off = 42.2575 units, p=0.219: While periostin's performance is not statistically significant, it remains a promising biomarker for Th2-driven asthma phenotypes, as shown in previous studies linking periostin to airway remodeling in Th2-high asthma (Fehrenbach, Wagner and Wegmann, 2017).

These biomarkers' overall poor diagnostic performance in ROC analysis indicates that they may not independently differentiate IgE-mediated asthma. However, their collective assessment alongside clinical parameters might improve diagnostic accuracy

Periostin could complement other biomarkers in identifying Th2-driven inflammation. SAA1 and IL-17 might be better suited for identifying non-IgE-mediated inflammation, given their association with broader inflammatory processes. IL-5 remains a critical biomarker for eosinophilic asthma, often overlapping with IgE-mediated phenotypes.

Conclusions

&

Recommendations

Conclusion:

1- The age groups (9-12) years' high prevalence among asthmatic patients in the study, and the majority were male.

2-Most of asthmatic patients in the study group were urban residence and family history.

3- Significant association between increase level of IL-5 and priostin in esionphilic asthmatic patients.

4- Significant association between increase level of SAA1 and IL-17 in neutrophilic asthmatic patients.

5-Significant association between high FeNo level and serum level of SAA1 and IL17.

6-Sensitization to aeroallergens like Russian thistle, meadow foxtail and rough pigweed was common.

Recommendations

Based on the study's findings, the following recommendations:

1-Large sample size included in further study.

2-Regard to IL-5, IL17 as biomarkers in asthma phenotyping.

3-Regard to priostin and SAA1 as biomarkers in asthma phenotyping.

4-Study polymorphism of IL-5, IL-17 for their receptors.

5-Study of IgE receptor polymorphism

6-flowcytometry for cytological analysis of sputum.

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Appendix

BT LAB
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Human Serum amyloid A ELISA Kit

USER INSTRUCTION

Cat.No E1225Hu
Standard Curve Range: 0.1-35ug/ml Microgram /ml
Sensitivity: 0.043ug/ml
Size: 96 wells / 48 wells
Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
CV(%) = SD/mean x 100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Human Serum amyloid A (also known as SAA) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human SAA antibody. SAA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human SAA Antibody is added and binds to SAA in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated SAA antibody. After incubation unbound

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Human Interleukin 5 ELISA Kit

USER INSTRUCTION

Cat.No E0091Hu
Standard Curve Range: 3-900ng/L
Sensitivity: 1.52ng/L
Size: 96 wells / 48 wells
Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
CV(%) = SD/mean x 100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Human Interleukin 5 (also known as IL-5) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-5 antibody. IL-5 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL-5 Antibody is added and binds to IL-5 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-5 antibody. After incubation unbound

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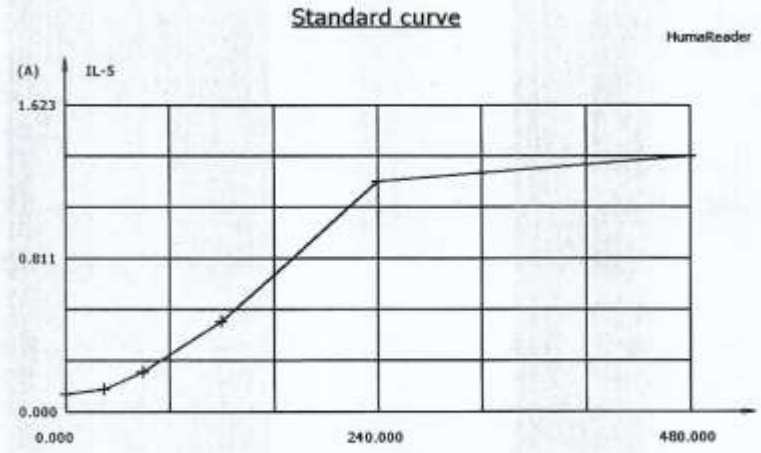

Program: IL-5 IL-5 Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6
Concentration(ng/l)	0.000	30.000	60.000	120.000	240.000	480.000
Abs(A)	0.093	0.119	0.207	0.473	1.213	1.352

Operator: Checker:
Print date: 04/22/2009

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Human Interleukin 17 ELISA Kit

USER INSTRUCTION

Cat.No E0142Hu

Standard Curve Range: 2-600ng/L

Sensitivity: 1.06ng/L

Size: 96 wells / 48 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Human Interleukin 17 (also known as IL-17) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-17 antibody. IL-17 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL-17 Antibody is added and binds to IL-17 in the sample.

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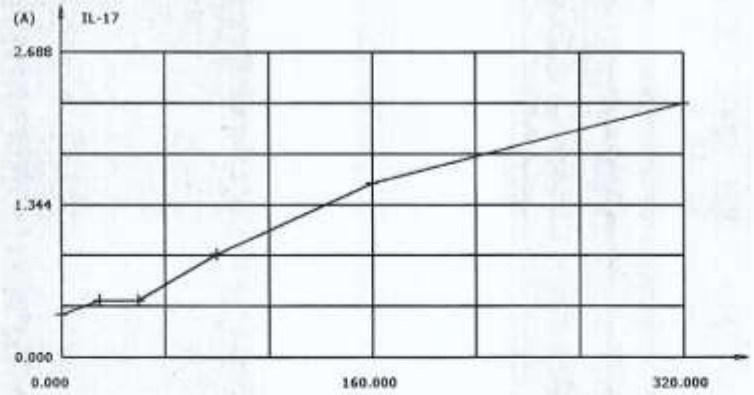
Standard curve

HumaReader



Program: IL-17 IL-17 Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6
Concentration(ng/l)	0.000	20.000	40.000	80.000	160.000	320.000
Abs(A)	0.370	0.493	0.494	0.897	1.533	2.240

Operator: Checker:
Print date: 04/22/2009

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Human Periostin ELISA Kit

USER INSTRUCTION

Cat.No E3226Hu
Standard Curve Range: 0.5-150ng/ml
Sensitivity: 0.251ng/ml
Size: 96 wells / 48 wells
Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
CV(%) = SD/mean x 100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Human Periostin (also known as POSTN) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human POSTN antibody. POSTN present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human POSTN Antibody is added and binds to POSTN in the

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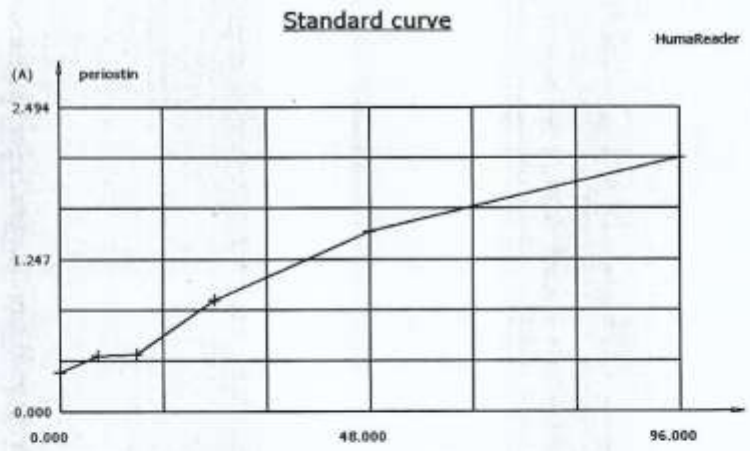
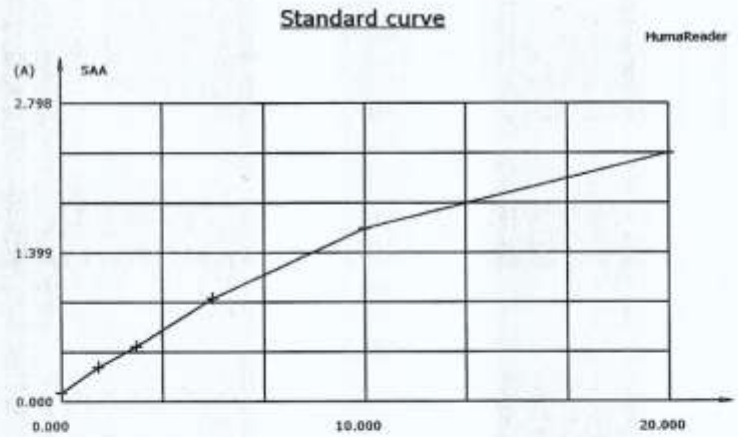


Program: periostin periostin Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6
Concentration(ng/ml)	0.000	6.000	12.000	24.000	48.000	96.000
Abs(A)	0.319	0.452	0.464	0.911	1.472	2.078

Operator: Checker:
Print date: 04/22/2009

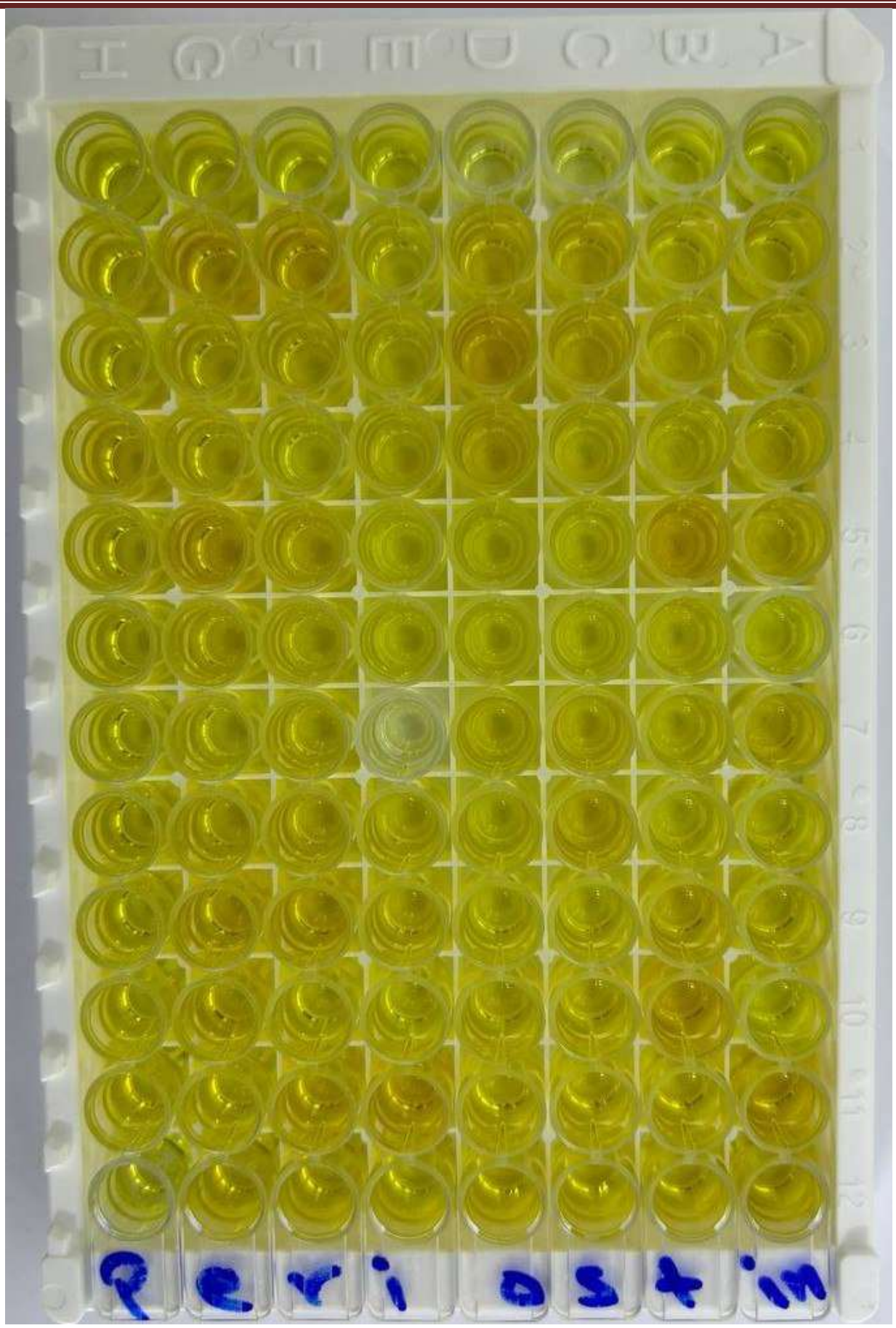


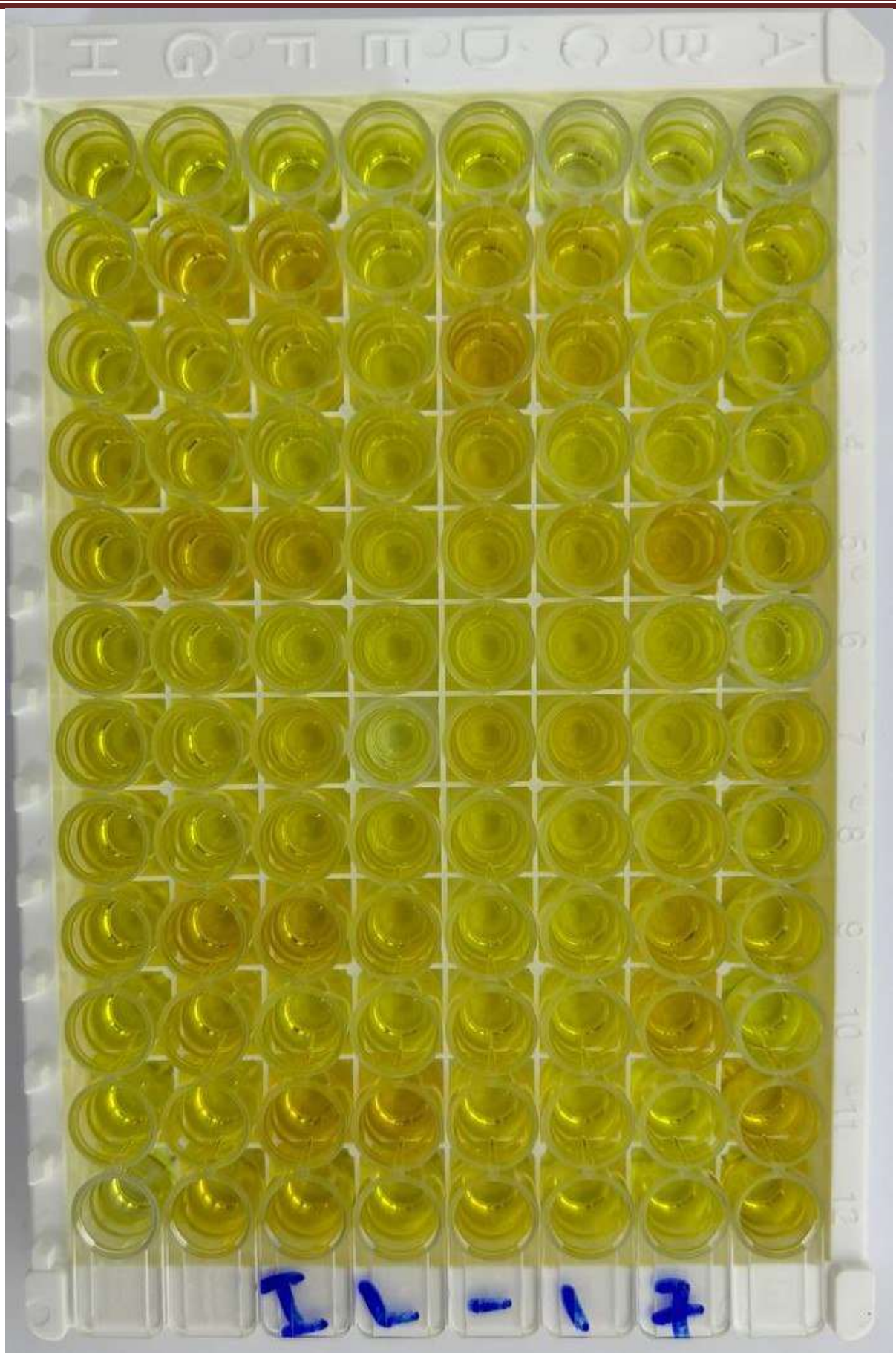
Program: SAA SAA Caculation: Curve Wavelength(nm): 450

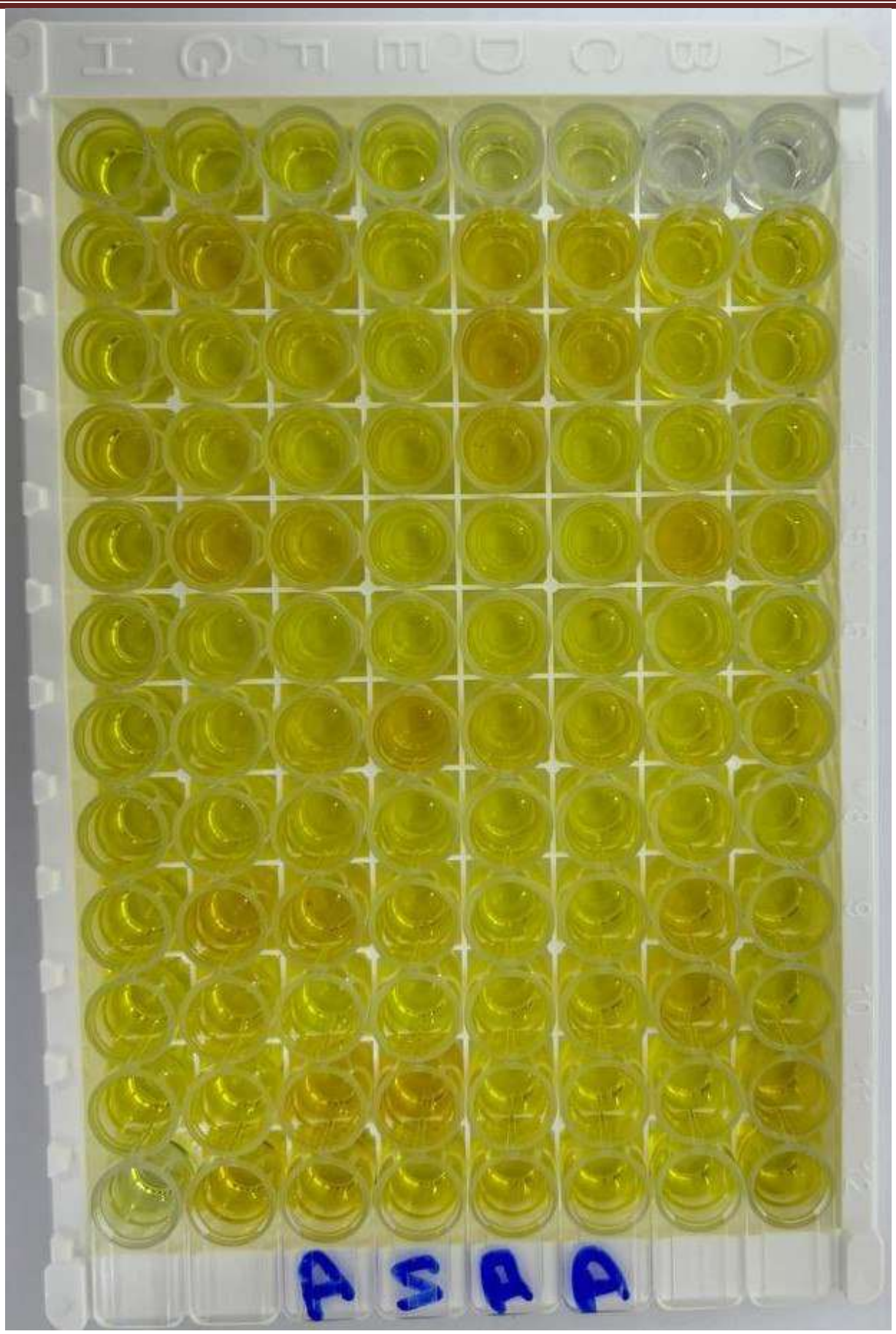
Standard sample	#1	#2	#3	#4	#5	#6
Concentration(ug/ml)	0.000	1.250	2.500	5.000	10.000	20.000
Abs(A)	0.083	0.323	0.512	0.964	1.624	2.331

Operator: Checker:
Print date: 04/22/2009


TL-5


Resazurin


IL-17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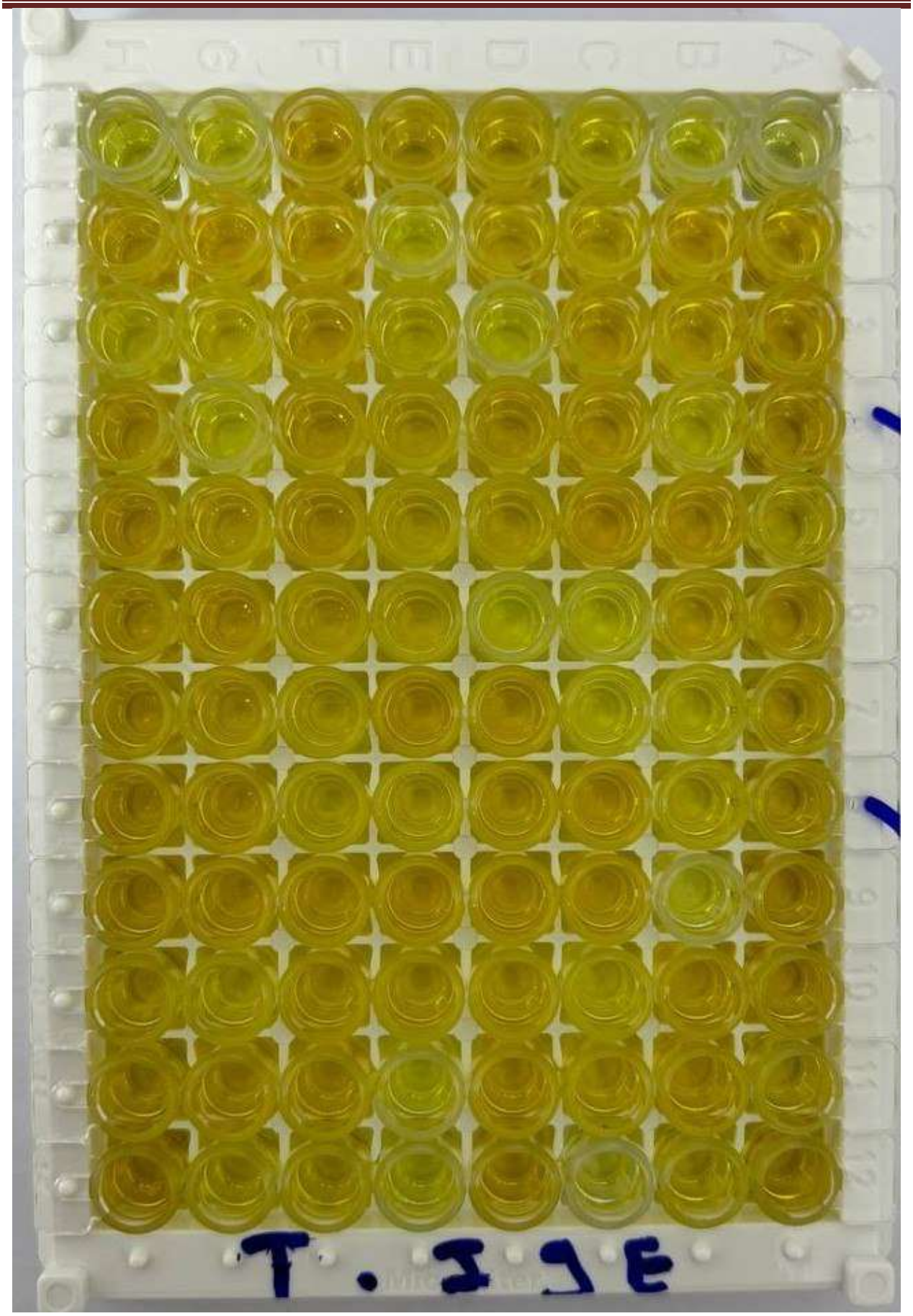
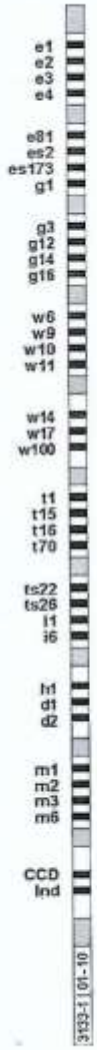


AG

The test strips include the following allergens:



Position	Allergen code	Allergen name
1	e1	Cat
2	e2	Dog
3	e3	Horse
4	e4	Cow
5	e81	Sheep
6	es2	Feather mix 1 (e85, e86, e111)
7	es173	Cage bird mix 3 (e78, e90, e91, e113)
8	g1	Sweet vernal grass
9	g3	Orchard grass
10	g12	Cultivated rye
11	g14	Cultivated oat
12	g16	Meadow foxtail
13	w6	Mugwort
14	w9	English plantain
15	w10	Goosefoot
16	w11	Russian thistle
17	w14	Rough pigweed
18	w17	Firebush (Kochia)
19	w100	Sorrel
20	t1	Box elder
21	t15	White ash
22	t16	White pine
23	t70	Mulberry tree
24	ts22	Tree mix 4 (t8, t11, t12, t14, t23)
25	ts26	Tree mix 6 (t2, t3, t4, t7, t9, t11)
26	i1	Honey bee venom
27	i6	Cockroach, German
28	h1	House dust
29	d1	Dermatophagoides pteronyssinus
30	d2	Dermatophagoides farinae
31	m1	Penicillium notatum
32	m2	Cladosporium herbarum
33	m3	Aspergillus fumigatus
34	m6	Alternaria alternata
35	CCD	CCD marker
a	Ind	Indicator band

ASTHMA QUESTIONARE

Name NO.

Age date of birth / /

Sex : male Female

Weight Height BMI

Residents : rural urban

Pt. hx of :eczema yes no

Urticaria : yes no

allergic rhinitis : no yes seasonal persistent

food allergy :no yes type

drug allergy: no yes type

family hx of :asthma No Yes Father

Mother Brother GF GM others

eczema: yes no

allergic rhinitis: no ◯ yes seasonal ◯ persistent ◯

Hx of food allergy: no ◯ yes type ☐

Pets at home : no ◯ yes type ☐

2nd hand smoking: yes ◯ no ◯

Age of symptom onset: ☐

Duration of asthma: ☐

Asthma severity:

Mild ◯ moderate ◯ severe ◯

Co-morbid: GERD ◯ sinusitis ◯

Asthma control:(last 4 weeks)

Well controlled ◯ partial controlled ◯

uncontrolled ◯

School hx.: absences no ◯ yes no. of days ◯

Performance good ◯ bad ◯

Exercise limitation: yes ◯ no ◯

Hx of exacerbation: no ◯ yes (/year)

Treatment: ICS ◯ SABA ◯ LABA ◯ LTRA ◯

OCS ◯ NONE ◯

Types of ICS : beclo ◯ budesonide ◯ fluticasone ◯

Types of LABA : salmetrol ◯ formetrol ◯

Dose: ☐

Duration of ICS use : ☐

Device: nebulizer ◯ DPI ◯ MDI ◯ spacer ◯ mask ◯

SABA use before exercise: yes ◯ no ◯

PFT result: FVC ☐ FEV1 ☐ FEV1/FVC ☐

FEF 25-75 ☐

FeNO level: ☐

WBC count: ☐

peripheral blood eosinophils :

blood neutrophils :

Total IgE :

Specific IgE:

PERIOSTIN protein level :

IL5:

IL17:

Serum amyloid A1 level:

ملخص

الربو هو اضطراب مزمن يصيب الشعب الهوائية ويتسم بالتهاب مجرى الهواء الذي يمكن عكسه كليًا أو جزئيًا، وهو عبارة عن فرط نشاط مجرى الهواء الذي يُعرَّف بأنه انكماش مجرى الهواء استجابة لمجموعة متنوعة من المحفزات. والأنماط الظاهرية الشائعة هي الربو التحسسي؛ والربو غير التحسسي؛ والربو الذي يصيب البالغين (يبدأ متأخرًا)؛ والربو مع تقييد مستمر لتدفق الهواء؛ والربو مع السمنة.

هدفت هذه الدراسة إلى التعرف على أنواع الربو وفقًا لنتائج المؤشرات الحيوية المناعية IL5 و IL17 و وبروتين البيريوستين وأميلويد المصل A1 (SAA1) في مرضى الربو الأطفال

منهجية الدراسة: تصف دراسة مقطعية عن الربو لدى الأطفال الذين تتراوح أعمارهم بين 5 و 15 عامًا، أجريت في مركز أمراض الرئة للأطفال في مستشفى كربلاء التعليمي، العراق، من أكتوبر 2023 إلى يوليو 2024. كان عدد المشاركين 100 طفل مصاب بالربو، مقسمين إلى أربع مجموعات (eosinophilic، neutrophilic، mixed granulocyte، allergic) بناءً على المؤشرات المناعية. تم استبعاد المرضى الذين يتناولون الكورتيكوستيرويدات الجهازية، أو المصابين بأمراض المناعة الذاتية، أو التهابات الجهاز التنفسي العلوي / السفلي. الاختبارات التي أجريت على عينات الدم التي تم تحليلها للمؤشرات الحيوية المناعية (IL-5 ، IL-17، periostin، SAA1، و IgE) باستخدام ELISA.

النتائج: كانت غالبية المرضى من الذكور (75%) وتتراوح أعمارهم بين 9 و12 عامًا (58%). وكان السكن الحضري هو السائد (65%). وكان التاريخ العائلي للإصابة بالربو مرتبطًا بشكل كبير (70%). كان لدى المجموعة eosinophilic مستويات أعلى من IL-5 وperiostin ، مما يشير إلى eiosinophil inflammation. كان لدى المجموعة neutrophilic مستويات SAA1 و IL-17 مرتفعة بشكل كبير، مرتبطة بالتهاب مجرى الهواء. أظهرت النمط الظاهري الحبيبي المختلط أنماط التهابية مميزة. وشملت المواد المسببة للحساسية الهوائية الشائعة نبات الشوك الروسي (22%) وقشرة القطط (18%). كانت مستويات IL-5 مرتفعة بشكل كبير في بعض المجموعات الإيجابية للمواد المسببة للحساسية الهوائية، على سبيل المثال، الحميض والرماد الأبيض.

لوحظت ارتباطات إيجابية بين IL-5 و IL-17و SAA1و Periostin. والعلامات الحيوية الأخرى. أظهر تحليل الخصائص التشغيلية للمستقبل (ROC) لـ IL-5 و Periostin إمكانية التنبؤ بالربو لدى المرضى الإيجابيين لمسببات الحساسية الهوائية.

تؤكد هذه الدراسة الشاملة على تباين أنماط الربو، وتحديد العلامات الحيوية الرئيسية ومسببات الحساسية الهوائية ذات الصلة بالتشخيص وأساليب العلاج الشخصية.

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قسم الأحياء المجهرية

دور المؤشرات الحيوية المناعية الانترلوكينات-5 ،17 ، بروتين البريوستين ، مصل الامايلويد أي-1 في تحديد النمط الظاهري لمرض الربو عند الاطفال

رسالة مقدمة الى
مقدمة إلى مجلس كلية الطب جامعة كربلاء استكمالاً جزئياً لمتطلبات الحصول على درجة الماجستير في الأحياء المجهرية الطبية

من قبل الطالب
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إشراف

الأستاذ الدكتور
الاء سعد حنفوش
2024

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