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Correlation of the Serum Interleukins 15, 22 and 23 with Severity of Pediatric Asthma

A thesis

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"Correlation of the Serum Interleukins 15, 22 and 23 with Severity of Pediatric Asthma"

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"Correlation of the Serum Interleukins 15, 22 and 23 with Severity of Pediatric Asthma"

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

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

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Dedication

This thesis work is dedicated

To the soul of my first inspiration … ***my Father***

To the guiding light of my life … ***my Mother***

To the support of life paths ***Brothers and Sisters*** *especially those who are not Blood-related who have been a constant source of support and encouragement during the challenges of this thesis and life*

To all my respected teachers

To everyone who gave me the hand of help or support when it was needed.

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Summary

Asthma is a common chronic inflammatory disease of the lungs airways, considered a major global health problem, affecting at least 3.5-20 % of the population in the world. Asthma affects people of all ages, but it usually begins in childhood. The inflammatory response is mediated by the allergic IgE produced during allergic sensitization, and linked to mast cells triggered by allergy re-exposure. IgE plays an active part in the pathogenesis of allergic asthma. It was suggested that IL-15 is critical for preservation of allergen induced airway hyperresponsiveness (AHR). While IL-22 plays a crucial role in antigen sensitization in a murine asthma model. Also, it was also suggested that the serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children.

This study is a case-control study that included 60 asthmatic children (35 male and 25 female) as the patients group age ranged between 4 months to 14 years old attending the asthma clinic at Karbala Teaching Hospital for Children in the period extending from November 2020 to March 2021, and 60 non-asthmatic (32 male and 28 female) age-matched patients group attending the outpatient clinic were recruited as control group.

Whole blood was collected from each participant, noting that the sera were used to determine total serum IgE and interleukins 15,22 and 23 levels for all samples by ELISA technique, while whole blood was used for absolute eosinophils count determination Using Sysmex XN-350 five differential automated hematology analyzers used to detect eosinophils count.

The result of this study showed a significant association between the IgE and IL-23 serum levels with asthmatic patients ($P=0.005$, $P=0.008$) respectively. Also, the study showed a significant difference between mild and moderate asthma regarding aggravating by upper respiratory inflammation ($P = 0.031$). In addition, there was a significant difference ($P = 0.049$) regarding the treatment between mild and moderate asthmatic patients.

Regarding the correlation between the studied markers and the duration of asthma, there was a significant negative correlation (*p = 0.030*) between IL-23 serum level and duration of asthma.

The present study showed a significant difference (*P* =0.046) in the severity of asthma depending on the duration, which was higher in moderate asthma than the mild asthmatic patients with a mean (36.70) and (17.86) respectively. There was a significant difference (*P= 0.004*) in the EOS count according to each asthma phenotype in the current study; it was the highest in allergic asthma with a mean (1.2518).

The results of this study have shown an elevated serum level of IL-23 in the patients group compared wthe ith control group of asthmatic children; therefore, IL-23 marker may have an important role in diagnosis of severe asthmatic children. Also this result shown a non-significant association between IL-15 and IL-22 with the severity of asthmatic children.

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List of Abbreviations

Abbreviation	Meaning
AHR	Airway Hyperresponsiveness
ASMCs	Airway Smooth Muscle Cells
APCs	Antigen Presenting Cells
Cyslt1r	Cysteinyl Leukotrienes Receptor
DC	Dendritic Cell
DCS	Dendritic Cells
FVC	Forced Vital Capacity
GM-CSF	Granulocyte–Macrophage Colony Stimulating Factor
Ig	Immunoglobulin
ICS	Inhaled Corticosteroids
ILCs	Innate Lymphoid Cells
IL	Interleukin
LT	Leukotrienes
NK	Natural Killer
NOD	Nucleotide-Binding Oligomerization Domain
OA	Occupational Asthma
PGD2	Prostaglandin D2
SLPI	Secretory Leukocyte Protease Inhibitor
SMC	Smooth Muscle Cells
Th2	T-Helper 2
TSLP	Thymic Stromal Lymphopoietin
TLRs	Toll-Like Receptors
TGF	Transforming Growth Factor
VCAM-1	Vascular Cell Adhesion Protein 1
WRA	Work-Related Asthma

Chapter One
Introduction and literature Review

1.1. Introduction

Asthma is considered an inflammatory disease in the airway, leading to airway hyper-responsiveness, obstruction, mucus hyper-production and airway wall remodeling (Kudo, Ishigatsubo and Aoki, 2013). In the development of this complicated illness, the interplay of hereditary and environmental variables is crucial (Mukherjee and Zhang, 2011). Chronic inflammation is associated with airway hyperresponsiveness (exaggerated airway-narrowing response to specific triggers such as viruses, allergens and exercise) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity(Vogelmeier *et al.*, 2017).

There are many cytokines implicated in the development of the chronic inflammatory processes that are often observed in asthma. Ultimately, these cytokines cause the release of mediators such as histamine and leukotrienes (LT), which in turn promote airway remodeling, bronchial hyperresponsiveness and bronchoconstriction. The CD4+ T-lymphocytes from the airways of asthmatics express a panel of cytokines that represent the Th2 cells(Ayakannu *et al.*, 2019).

Eosinophils, which tend to accumulate at sites of allergic inflammation, contribute to the development of bronchial asthma(Weller, 1997). The allergic asthmatic response to allergen exposure is associated with immunoglobulin E (IgE) mediated mast cell activation, resulting in the accumulation of leukocytes such as eosinophils and Th2 lymphocytes in the airway(Umetsu and DeKruyff, 1997; Rajakulasingam *et al.*, 1997).

The IL-15 has a potent inhibitory effect on the airway obstruction that occurs in response to environmental allergens (Venkateshaiah *et al.*, 2018). IL-22 is produced by Th17 cells at the site of allergic airway inflammation and attenuates eosinophilic inflammation and airway hyperresponsiveness (AHR), presumably by inhibiting cytokine and chemokine production from lung epithelial cells. In asthma patients, however, excessive production of IL-22 may

lead to the progression of airway remodeling by enhancing the proliferation and migration of airway smooth muscle cells (ASMCs) (Hirose, Takahashi and Nakajima, 2013). A study performed by Ciprandi *et al.* (2012) demonstrated that serum IL-23 was up-regulated in asthmatic children and changes well related to lung function improvement. Thus, it is presumable that IL-23 could be a suitable marker of allergic inflammation in asthma.

Aim of the study:

This study aimed to investigate the role of IL-15, IL-22 and IL-23 as immunological markers for disease severity among children with asthma disease, this was achieved by the following objectives:

1- Estimation of eosinophils count in patients and control by Sysmex XN-350 five differential automated hematology analyzers

2- Determination of IgE serum level in patients and control by ELISA test.

3- Determination of IL-15, IL-22 and IL-23 in patients and control by ELISA test.

4- Correlation of Immunological markers level with disease severity and treatment.

1.2. Literature Review

1.2.1. Asthma Definition: -

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils. It's the most prevalent child respiratory disease, affecting 235 million people worldwide (World Health Organization, 2021).

In susceptible individuals, the chronic inflammation causes symptoms that are usually associated with widespread but variable airflow obstruction that is often reversible, either spontaneously or with treatment, and causes an associated increase in airway responsiveness to a variety of stimuli'(Monstrey *et al.*, 1992). Asthma typically presents with 'wheezing', a high-pitched whistling sound heard on expiration, and, if the asthma is severe, also on inspiration. Asthma also causes shortness of breath and chest tightness and can cause cough, particularly in children (Asher and Pearce, 2014).

1.2.2. Epidemiology

Epidemiologic data have shown that over the last few decades, the prevalence of asthma has increased both domestically and globally (Masoli *et al.*, 2004; Moorman *et al.*, 2012;). Therefore, it is no surprise that asthma has become not only a major national health issue but also a significant global health issue. There is a limited epidemiology study on asthma prevalence and incidence in Baghdad, the prevalence of asthma was 22.3% in a study that included 3360 primary-school children.(Al-Thamiri, Al-Kubaisy and Ali, 2005). In the United Arab Emirate, the prevalence was 13% out of study included 3200 children. In the Kingdom of Saudi Arabia the prevalence increased significantly from 8% in 1986 to 23% in 1995 (Al-Maskari *et al.*, 2000; Al Frayh *et al.*, 2001).

A study from Italy has confirmed that asthma is on the rise in countries outside of the United States, as they showed an increase in asthma prevalence in

Italy from 3.4 to 7.2% over an approximately 25-year study period (Maio *et al.*, 2016). Interestingly, within developed countries, such as Australia, asthma is more common among the economically disadvantaged, yet in developing countries an asthma diagnosis is more common among the higher class.

Impact on age, 18.7 million of the 25.7 million persons with asthma in the United States in 2010 were adults and 7.0 million were children (Moorman *et al.*, 2012). Similarly, the National Health Interview Survey reported that 6.7 million or 9% of children ages 0–17 years in the United States had asthma in 2007, with a lifetime prevalence as high as 13% (Hill and Wood, 2009). The global prevalence of pediatric asthma is reported to be approximately 14%, and similarly to the adult group, is rising (Asher *et al.*, 2006).

Impact on race and ethnicity, the breakdown of race and ethnicity in asthma was reported by the CDC in 2002 with the following results: a 7.6% prevalence in the non-Hispanic White population, 9.3% in the Black population, 2.9% in the Asian population, 1.3% in the Native Hawaiian/Pacific Islander population, 11.6% in the American Indian/Alaska Native population, 5.0% in the Hispanic population, 15.6% in the Multiracial (non-Hispanic) population, and 7.2% in the Other (non-Hispanic) population (Control and Prevention, 2004).

More current numbers indicate that 19.1 million persons with asthma in 2010 were white and 4.7 million were black, while asthma prevalence was higher in black persons (11.2%) than in white persons (7.7%) (Moorman *et al.*, 2012).

1.2.3. Risk Factors

Childhood asthma and adult-onset asthma are known to share many of the same causes and triggers. While there is stronger evidence on the role of environmental factors as triggers than causes, there is increasing evidence for interactions among and between environmental and other intrinsic factors, such as genetics and atopy, to potentially cause asthma (Dharmage, Perret and

Custovic, 2019).The vast majority of childhood onset asthma manifests as an allergic phenotype, while there is a predominance of the non-allergic phenotype in adult-onset asthma. However, both allergic and non-allergic asthma can exhibit individual responses to both allergic and non-allergic airborne triggers such as animal hair and dander, pollen, and mold (fungal) spores, food allergens, tobacco smoke, or other pollutant exposures (Jacquemin *et al.*, 2012).

1.2.3.1. Parental and Personal Smoking

In utero maternal smoking and parental smoking in early life has been shown to be temporally associated with increased asthma in young children (Burke *et al.*, 2012). some evidence from multi-generational studies suggests that grandmaternal smoking while the mother is in utero and paternal smoking during his adolescence can independently increase the risk of subsequent offspring childhood asthma. These findings suggest that tobacco smoking may cause heritable modifications of the epigenome, which increase the risk of asthma in future generations (McCreanor *et al.*, 2007).

Smoking also seems to interact with sex. Female smokers had a higher prevalence of asthma than female non-smokers, but this difference was less frequent for males, suggesting that females may be more susceptible. Many studies have found that personal smoking predisposes an individual to increased risk of incident or new-onset asthma, although smoking-onset in adolescence, or adulthood typically occurs after early-onset asthma (McLeish and Zvolensky, 2010)

As non-atopic asthma becomes increasingly common compared with atopic asthma in adults, this is most likely because this phenotype frequently coincides with a substantial history of cigarette smoking and its potential to predispose to chronic airflow limitation (Zbikowski *et al.*, 2002; McLeish and Zvolensky, 2010; Perret *et al.*, 2016). Smokers with asthma form a distinct group

that are more likely to have suboptimal asthma control (McLeish and Zvolensky, 2010) and develop asthma-COPD overlap syndrome (Bousquet *et al.1990*) in later life, characterized by incompletely reversed airflow obstruction following an inhaled bronchodilator (Roman *et al.*, 2015).

From an epidemiological viewpoint, smoking is common in people with asthma, with around one-quarter of adults from 70 countries receiving recent asthma treatment also reporting to be current smokers (To *et al.*, 2012). Some evidence suggests that people with asthma may be more likely to smoke, and this was seen especially in adolescents who have more severe disease (Roman *et al.*, 2015).

1.2.3.2. Outdoor Air Pollutants

Outdoor air pollution almost certainly has a major global impact on asthma for children and adults, especially in China and India (Anenberg *et al.*, 2018). Worldwide, in 2015, 9–23 million and 5–10 million annual asthma emergency room visits have been attributed to the outdoor air pollutants ozone and particulate matter with an aerodynamic diameter <2.5 μm (PM2.5), respectively. (Exposure to PM1 has been found to increase the risk of asthma and asthma-related symptoms, especially among boys, and those with allergic predisposition (Anenberg *et al.*, 2018).

Residential markers of traffic-related air pollution, including nitrogen dioxide (NO2) exposure and distance to major roads, have been associated with increased risk for new-onset asthma, persistence of asthma and current asthma in a middle-aged, asthma-enriched, population-based cohort study (Bowatte *et al.*, 2017). In a natural experiment of 60 young to middle-aged adults with mild-to-moderate asthma, when compared with walking in the less polluted Hyde Park in London, walking along Oxford Street was associated with reductions in lung function, neutrophilic inflammation and airway acidification (McCreanor *et al.*,

2007). These changes were greater for individuals with moderate asthma compared with mild disease at baseline.

1.2.3.3. Outdoor Allergens

Exposure to ambient grass pollen is an important trigger for childhood asthma exacerbations requiring emergency department attendance (Erbas *et al.*, 2018). There is also scant evidence on the role of early life exposure to pollen in the development of childhood asthma (Erbas *et al.*, 2013). However, less evidence is available on the role of pollen in adult asthma (Guilbert *et al.*, 2018), except in "Thunderstorm asthma" which is related to a combination of factors.

In relation to other outdoor allergens, increasing evidence indicates that asthmatic children are susceptible to exacerbations that lead to hospitalization when exposed to outdoor fungal spores (Tham *et al.*, 2017). Furthermore, high concentrations of outdoor fungal/mold exposure on peak days have been linked to asthma exacerbation and mortality in adults (Packe and Ayres, 1985; Alderman, Sloan and Basran, 1986; O'hollaren *et al.*, 1991). IgE sensitization to fungal species is associated with increased asthma severity, neutrophilic inflammation, and reduced lung function consistent with Asthma-chronic obstructive pulmonary disease overlap syndrome (Bousquet *et al.1990* ; Fairs *et al.*, 2010).

1.2.3.4. Thunderstorm Asthma

Thunderstorm asthma is defined as epidemics that occur during or shortly after a thunderstorm, where individuals affected would experience asthma-related symptoms such as breathlessness, wheezing and coughing. "Thunderstorm asthma" (Andrew *et al.*, 2017; Thien *et al.*, 2018) is the outcome of a complex interaction between multiple factors but not necessarily any one of them individually. Under certain weather conditions such as a thunderstorm, pollen grains may swell and burst to form fine respirable particles that are sufficiently

small to enter the lower respiratory tract and precipitate severe asthma in those susceptible. This can occur in sensitized individuals who may or may not have a prior history of asthma or asthma symptoms, but who often have a history of allergic rhinitis. Fungal spore allergens may also be involved (Alderman, Sloan and Basran, 1986).

1.2.3.5. Indoor Environment

Indoor pollutants such as products of combustion, including Particulate matter (PM) and Nitrogen dioxide (NO2), and airborne allergens have been the subject of intense scrutiny as determinants of asthma.

There is substantial evidence to suggest that indoor allergens generated by house dust mite, mold and cat are triggers for both childhood and adult asthma, especially in those sensitized (Downs *et al.*, 2001; Salo *et al.*, 2006; Burr *et al.*, 2007). However, their role in the etiology of asthma is not clear. On the other hand, primary prevention trials on reduction of allergen exposure in early life have failed to detect any benefits. Some observational studies have even reported exposure to allergens in infancy may help develop tolerance and reduce the risk of asthma. However, the evidence is not consistent. Interestingly, there is increasing evidence on this tolerance hypothesis in the etiology of food allergy in which a clinical trial has shown that early consumption of peanuts can reduce the development of peanut allergy (Du Toit *et al.*, 2015; Gruchalla and Sampson, 2015). These findings suggest that it may be worth exploring this notion of early exposure to allergens leading to development of tolerance, which in turn may reduce the risk of developing asthma.

1.2.3.6. Occupational Exposures

Occupational exposures to asthma antigens or inciting sensitizing agents are common and often under-recognized causes of work-related asthma (WRA). WRA includes two distinct subtypes: work-aggravated/exacerbated asthma

(WEA) occurring in individuals with pre-existing asthma, and occupational asthma (OA) occurring in individuals without previous asthma. OA is typically subclassified into immunoglobulin (Ig)-E-mediated or sensitizer-induced OA (90%) and irritant-induced occupational asthma (10%) (Vandenplas, Suojalehto and Cullinan, 2017). A diagnosis of WRA requires the objective diagnosis of asthma with symptoms temporally related to the individual's place of employment (Beach *et al.*, 2007). Over 250 agents may potentially cause sensitization and possibly (Malo and Chan-Yeung, 2009;; Baur and Bakehe, 2014; Tarlo and Lemiere, 2014; Crewe *et al.*, 2016).

Differentiating sensitizer-induced OA from WEA can be a major challenge for managing clinicians. The time-to-diagnosis of sensitizer-induced OA varies but is usually made between 2 and 4 years following the onset of work-related symptoms, and this timeframe is substantially shorter for the diagnosis of WEA as these individuals are usually medically managed for pre-existing asthma (Santos *et al.*, 2007). Among compensation claims, confirmed OA diagnoses most have a causative sensitizing agent identified (Tarlo *et al.*, 1995). Despite challenges in estimating the true incidence of OA, around 10–20% of all adult-onset asthma is thought to be caused by respiratory sensitizers and/or irritants in the occupational setting.

1.2.3.7. Lifestyle Factors

Although already mentioned as an “asthma-plus” co-morbidity, the prevalence of obesity in countries in which a Westernized diet predominates is now of epidemic proportions. These dietary patterns feature a high calorie intake which is high in saturated fat and refined sugars and associated with a high glycaemic index, as well as low nutritional value in terms of dietary fiber and vitamins. While this “obesogenic diet” may lack antioxidant and anti-inflammatory properties (Wood, 2017), a meta-analysis has found being

overweight and obese to be associated with a dose-response increase in incident asthma in adults (Beuther and Sutherland, 2007). Female obesity was found associated with a pauci-eosinophil and non-atopic asthma endotype that is symptom-predominant and less steroid-responsive in previous cluster and Latent class analysis LCA (Haldar *et al.*, 2008; Jeong *et al.*, 2017). For all individuals with otherwise poorly controlled asthma, the behavior of avoiding strenuous exercise might confuse severe disease with well-controlled asthma, and this in turn can lead to poorer fitness levels and a propensity to weight gain (Bateman *et al.*, 2008). This is of particular importance to children with asthma, at a time when lifestyle patterns are being especially shaped by external factors.

1.2.4. Pathogenesis

Chronic inflammatory disease of the airways in most patients with asthma is characterized by eosinophilic hyper-infiltration, overproduction of mucus in airways, hyper-reactivity and eventually remodeling of airways. Pathological changes in asthma are heterogeneous and these non-specific changes are mostly induced by T-helper 2 (Th2) cells and their related-cytokines including Interleukins (IL) 4, 5 and 13 (Larché, Robinson and Kay, 2003). In addition to eosinophils, in other asthma phenotypes like severe asthma, neutrophils can also accumulate in the airways (Louis *et al.*, 2000). Depending on the number of various cells in the sputum induced by hypertonic saline, asthma can be classified into four phenotype: eosinophilic asthma (more than 1% sputum eosinophils), neutrophilic (sputum neutrophils more than 60%), mixed granulocytic asthma (both neutrophils and eosinophils increase in number) and asthma with normal sputum eosinophil and neutrophil which is also referred as pauci granulocyte (Simpson *et al.*, 2006; Porsbjerg *et al.*, 2009).

1.2.5. Role of innate immunity

The innate immune system comprises a range of host defense systems that generate nonspecific responses to environmental triggers. It encompasses cellular and noncellular components, table 1-1. The airway epithelium and mucosal layer also provide innate immune functions beyond serving as mechanical barriers. Innate immune cells include dendritic cells, innate lymphoid cells (ILCs) of which there are several types (Philip and Artis, 2013), and leukocytes such as macrophages, neutrophils and eosinophils. Pattern recognition receptors such as Toll-like receptors (TLRs), retinoic acid inducible gene I (RIG-I)-like receptors and nucleotide-binding oligomerization domain (NOD)-like receptors recognize different ligand motifs and are important in mediating early immune responses that subsequently shape adaptive immunity. Noncellular components of innate immunity are also important in host defense and include a variety of secreted factors such as lactoferrin, interferons, defensins, secretory leukocyte protease inhibitor (SLPI) and LL-37 (cathelicidin), all of which have antimicrobial effects (Morris *et al.*, 2013).

Table (1.1): Components of innate immunity likely to mediate airway microbial–immune interactions in asthma

	Cellular	Soluble
Epithelium	Granulocytes	Antimicrobial peptides
Airway mucus	Macrophages	Lactoferrin
	Eosinophils	Interferons
	Neutrophils	Defensins
	Basophils	LL-37 (cathelicidin)
		Secretory leukocyte protease inhibitor
	Other cell types	

	Dendritic cells	
	Innate lymphoid cells	
	Group 1 (natural killer cells)	
	Group 2 (natural helper cells)	
	Cellular receptors	
	Toll-like receptors	
	NOD-like receptors	
	RIG-I like receptors	

The respiratory tract is continually exposed to airborne matter, which translates into frequent opportunity for interaction with mucosa-associated immunity. In addition to air, aspirated material from the upper respiratory or gastrointestinal tracts also serves as potential sources of immune stimuli, including microbes and bile salts. The mucus-lined epithelium, dendritic cells with their interdigitating processes that contact the airway lumen, and secreted antimicrobial peptides, all are involved in the initial contact and responses to external triggers (Huang, 2015).

In addition to being an anatomic barrier, the airway epithelium is an important coordinator of immune function (Hallstrand *et al.*, 2014). Secretory epithelial cells produce antimicrobial peptides, including lysozyme, defensins and IgA (Fahy and Dickey, 2010). Epithelial cells also shape type 2 mucosal immune responses, through their production of thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. These epithelial cells derived cytokines stimulate other cell types to promote downstream Th2 inflammation. The potential clinical relevance of epithelial-driven Th2 responses was demonstrated in a study of TSLP inhibition in asthma (Gauvreau *et al.*, 2014). Treatment with an anti-TSLP

antibody attenuated allergen-induced bronchoconstriction and decreased markers of Th2-related airway inflammation such as sputum eosinophils and fraction of exhaled nitric oxide. Other cytokines produced by epithelial cells include IL-10 and transforming growth factor (TGF)-beta, which regulate the activity of other immune cells. The airway epithelium therefore is adynamic orchestrator of immune defense, straddling innate and adaptive immunity (Huang, 2015b).

Airway mucus is a complex entity with important properties beyond its contribution to normal mucociliary clearance (Fahy and Dickey, 2010). Mucus gel is composed of mostly water and nonaqueous components such as mucins and nonmucin proteins. Mucins are very large glycoproteins, the predominant types found in human airways being MUC5AC and MUC5B. Recent evidence in mice suggests an interesting and critical role for MUC5B, but not for MUC5AC, in airway defense.A study found that the absence of MUC5B leads to lung inflammation, impaired immune homeostasis and chronic infection with multiple bacterial species (Roy *et al.*, 2014). In addition, antimicrobial proteins are present in airway mucus, wherein microorganisms can become trapped and either are removed by normal mucociliary clearance or propagate (Roy *et al.*, 2014).

Dendritic cells are also an important bridge between the innate and adaptive immune systems, including the development of allergic inflammation and responses to viruses (van Helden and Lambrecht, 2013; Kim and Lee, 2014). As antigen-presenting and processing cells, they present fragments of microbes that can initiate a broad range of regulatory and adaptive T-cell responses, including Th1, Th2, Th17 pathways. The programmatic breadth of dendritic cells, in addition to the epithelium, enables them to be critical in activating a range of immune cell types, including T cells and ILCs (van Helden and Lambrecht, 2013).

ILCs comprise a family of immune cells present at barrier surfaces and have the capacity to respond quickly to environmental signals, including commensal bacteria. Although they do not express classic lineage markers, ILCs can manifest a diverse repertoire of responses. In particular, Group 1 ILCs, also known as conventional natural killer cells, play a role in antiviral responses, while Group 2 ILCs, or natural helper cells, produce IL-4, IL-5 and IL-13. As with dendritic cells, microbiota can engage ILCs leading to multiple pathways of immune activation that are not necessarily mutually exclusive (Hansel, Johnston and Openshaw, 2013; Philip and Artis, 2013).

Finally, the influx of leukocytes into bronchial tissue is a histologic hallmark of airway inflammation seen in asthma. Phagocytic macrophages have an important role in host defense against pathogens, but granulocytic eosinophils and neutrophils are often increased in sputum samples from asthmatic individuals. Importantly, the relative prevalence of sputum eosinophils varies by patient, by asthma phenotype and even over time. Although eosinophilic inflammation is often observed, many other asthmatic individuals do not consistently demonstrate, if at all, airway eosinophils in sputum Thus, 'no-eosinophilic asthma' characterizes a significant and important proportion of patients who tend to be less responsive to current therapies, the majority of which target aspects of Th2 inflammation. (McGrath *et al.*, 2012).

1.2.6. Role of adaptive immunity

Asthma was classified as an allergic inflammatory disease, characterized mainly by eosinophilia and immunoglobulin E (IgE), associated with airway hyperresponsiveness, smooth muscle cells (SMC) hypertrophy, airway remodeling and increased mucus production. The immune recognition of environmental allergens is initiated by antigen presenting cells (APCs), such as dendritic cells (DCs) (but also B lymphocytes, and many other cell types); these

will induce the maturation of T-naïve cells into Th-2 cells, able to produce cytokines such as IL-4, IL-5 and IL-13 (Lambrecht and Hammad, 2015; Peebles and Aronica, 2019). IL-4 produced by Th-2 cells activates B cells to differentiate into plasma cells and switching antibody production to the IgE isotype (Delphin and Stavnezer, 1995). The cross-linked binding of allergen-specific IgE molecules, the allergen itself and effector cells like mast cells and basophils, determines the degranulation of preformed (i.e: histamine, tryptase) and other mediators (i.e.: leukotriens, prostaglandings) contributing to the development common symptoms of asthma (Lambrecht and Hammad, 2015; Peebles and Aronica, 2019). Moreover, IL-4 contributes to polarize T-naïve cells to the Th2 cells, therefore further enhancing the entire Th-2-mediated inflammatory response (Masinovsky, Urdal and Gallatin, 1990; Swain, 1995). Other relevant activities of IL-4 in the asthma pathogenesis are the induction of Vascular cell adhesion protein 1 (VCAM-1) expression, which contributes to direct the migration of eosinophils, basophils, monocytes and T cells to the site of allergic inflammation (Masinovsky, Urdal and Gallatin, 1990), and the induction of mucin gene expression with subsequent increase in airway mucus production (Xia *et al.*, 2017).

Broncho-alveolar lavage (BAL) studies on asthmatics revealed that IL-4 is central in the immunopathogenesis of allergic phenotypes of asthma (Robinson *et al.*, 1992; Virchow *et al.*, 1994), while its mRNA expression was not found in nonallergic asthmatics (Virchow *et al.*, 1994); interestingly, nonallergic but eosinophilic asthmatics had elevated levels IL-5 (as it happens also in allergic patients) suggesting its crucial role in determining eosinophilic airway inflammation even in absence of allergic stimulation (Virchow *et al.*, 1994).

Another mechanism of upregulations of mast cells was described and it passes through the production of IL-9 by both Th-2 and ILC-2 cells (Yao *et al.*, 2013). Another adaptive immune system mechanism that seems to be implicated

in the pathogenesis of asthma, mainly neutrophilic phenotypes(Chang *et al.*, 2017; Ricciardolo *et al.*, 2017), is IL-17 (IL-17A and IL-7 F) as the pivotal cytokine. Th-17 cells, other T cells, NK cells, ILC-3 and mast cells express this cytokine. With an abundance of IL-17A and IL-17 F, neutrophil influx is regulated indirectly through the induction of airway epithelial cells and stromal cell production of cytokines, which influence the chemotaxis of neutrophils to the scene. Relating back to neutrophilic asthma pathogenesis, IL-17 is the dominant signaling molecule that elicits the activation of its receptor and it is expressed on smooth muscle cells, inducing smooth muscle cells hypertrophy (Willis *et al.*, 2015).

IL-17 receptor is also expressed on epithelial fibroblast, macrophages and endothelial cells, contributing to explaining the potential role of IL-17 in airway remodeling (Ota *et al.*, 2014). Although the mechanism is still unclear, researchers believe that cytokines released by neutrophils such as IL-6, transforming growth factor beta, IL-1beta, tumor necrosis factor alpha, IL-21 and IL-23 in variable combination, can activate key transcription factors such as STAT3 and RORC2 to induce the differentiation of naïve CD4+ T cells to Th-17 cells (Nalbant and Eskier, 2016).

1.2.7.Role of IgE

The biological role of IgE is complex and related to its ability to influence the functioning of several immune and structural cells involved in the pathogenesis of chronic allergic inflammation. The biological pathways that IgE uses to influence cell activity rely on interactions with specific receptors. Two classes of receptors have been identified: high-affinity (FcεRI) receptors and low-affinity CD23 (or FcεRII) receptors. FcεRI receptors are not only expressed by mast cells and basophils but also by dendritic cells (DCs), airway smooth muscle cells (ASMCs), epithelial cells, endothelial cells, and eosinophils(Maurer *et al.*,

1996; Sihra *et al.*, 1997; Kraft and Kinet, 2007; Redhu and Gounni, 2013). DCs can provide all of the co-stimulatory signals required for activation of T cells, which play a key role in the pathogenesis of Biliary atresia (BA).

DCs are dedicated antigen-presenting cells and are key in the induction of Th2 cell activation in the primary immune response to allergens (Gaurav and Agrawal, 2013; Froidure, Shen and Pilette, 2016). The ability to present antigens is amplified by IgE bound to FCεRI receptors expressed on the surface of DCs. It has been shown that IgE captures the allergens, facilitating their presentation to memory Th2 lymphocytes (Schroeder *et al.*, 2010). FcεRI–IgE-dependent allergen presentation by DCs may critically lower the atopic individual's threshold to mount allergen-specific T cell responses. In fact, the targeting of allergens to FcεRI via IgE leads to a 1000-fold increase in the activation of T cells in addition to the production of chemokine ligand 28 (CCL28), a chemokine that selectively attracts Th2 lymphocytes (Khan and Grayson, 2010).

Furthermore, the activation of allergen-specific Th2 cells is associated with an amplification of allergen-specific IgE production in a vicious cycle of the pathogenic mechanisms of allergic asthma. IgE antibodies are also able to negatively modulate the innate function of plasmocytoid DCs. In these cells, the activation of FcεRI receptors blocks, or at least reduces, the intracellular signals involved in type I interferon (IFN) production (Lynch *et al.*, 2014). The reduction in IFN production correlates with the defect in anti-viral response in allergic asthma patients (Kelly and Busse, 2008). Moreover, viral respiratory infections can be the initial cause of asthma, and can result in exacerbations and worsen severity. Research to understand the relationship between IgE and virus infection susceptibility was spurred, in part, by the results of the clinical trial performed using omalizumab in children with asthma. The study found that anti-IgE therapy was able to reduce the exacerbations typically occurring in the spring but mainly in the fall when the children go back to school. It is known that the majority of

the exacerbations are induced by viral respiratory infections (Busse *et al.*, 2011). The relationship between virus and IgE is confirmed by the fact that respiratory syncytial virus, parainfluenza virus, Ebstain-Barr virus are able to induce a specific IgE antibody response .(Welliver *et al.*, 1982; Russi *et al.*, 1993; Smith-Norowitz *et al.*, 2011) Furthermore, the mechanisms by which IgE antibodies and viral infection may interact during asthma exacerbations could be related to the up-regulation of the γ chain of FcεRI receptors induced by IFN (Holt and Sly, 2012).

The importance of B lymphocytes to present antigens for antibody production is well documented and IgE fixed to the membrane through CD23 molecules (IgE low-affinity receptors) increases their capacity to capture allergens, amplifying the allergic response .(Dullaers *et al.*, 2017; Gavala *et al.*, 2020) In addition, some very interesting data have suggested that omalizumab may modulate human B-cell functions, including IgE synthesis (Chan *et al.*, 2013).

Among structural cells, airway smooth muscle cells (ASMCs) have been considered for many years to only be involved in bronchoconstriction during acute exacerbations of asthma (Redhu and Gounni, 2013) . IgE directly activates ASMCs to produce cytokines [IL-4, IL-5, IL-13, tumour necrosis factor (TNF)-α, thymic stromal lymphopoietin (TSLP)], chemokines (CCL5, CCL11, CXCL8, CXCL10) and the traditional mediators, and cause ASMC proliferation and contraction(Ferreira *et al.*, 2018) . The activation of ASMCs leads to their hypertrophy and hyperplasia, both of which correlate with asthma severity (Romagnani, 2004). Furthermore, upon IgE-driven stimulation, ASMCs also produce and secrete extracellular matrix proteins that are key factors involved in airway wall remodeling. These effects represent the biological background that explains why anti-IgE mAbs prevent extracellular matrix and collagen deposition, and airway remodeling(Roth *et al.*, 2013).

The IgE biological network also includes effects on airway epithelial cells that express low-affinity IgE (CD23) receptors, which are involved in the transport of IgE-allergen complexes across the polarised airway mucosal barrier (Palaniyandi *et al.*, 2011). Since epithelial cells are the first cells to be exposed to inhaled allergens, they play a key role in the initiation of allergic airway inflammation, and several studies have identified an important role of the airway epithelial-derived cytokines, IL-25, IL-33, and TSLP in asthma pathogenesis (Mitchell and O'Byrne, 2017). Overall, the wide range of functions of IgE place it at the center of the pathogenic mechanisms of the allergic inflammatory process.

1.2.8. Role of eosinophils

Eosinophils were first identified in the late nineteenth century and eosinophilia has been known to be associated with a wide variety of conditions, including asthma and atopic diseases (Kouro and Takatsu, 2009). The relatively discovery of IL-5, in 1980(Takatsu, Tominaga and Hamaoka, 1980), its interaction with eosinophils, and subsequent results of anti-IL-5 blocking mAb treatment in patients with asthma confirmed the importance of IL-5 in eosinophil-mediated inflammation in humans (Leckie *et al.*, 2000).

Increased numbers of eosinophils have been reported in the peripheral blood of patients with eosinophilic disorders such as asthma. Eosinophilic inflammation of the airways characterizes disease severity in subsets of individuals with severe asthma and there is a direct relationship between eosinophil count and the frequency of asthma exacerbations(Bousquet *et al.*, 1990; Leckie *et al.*, 2000; Kips *et al.*, 2003). Eosinophil differentiation, activation and survival mainly depends upon the effects of IL-5. This cytokine, produced at the bronchial level by Th2 cells as well as by mast cells and basophils, circulates through the blood and arrives at the bone marrow where it stimulates eosinophil

progenitors, which migrate towards the bronchial walls under the effect of chemokines such as eotaxins (CCL11)(Kita, 2011) . However, other cytokines are also able to directly influence eosinophils. Granulocyte–macrophage colony stimulating factor (GM-CSF) and IL-3 activate and enhance eosinophil functions, such as cytotoxic killing, superoxide production, leukotriene production, and Ig-induced degranulation (Nagata *et al.*, 1999). In addition, it should be underlined that eosinophils are greatly influenced by prostaglandin D2 (PGD2) in that they express, like Th2 cells and basophils, the specific prostaglandin D2 receptor 2 (DP2 or chemoattractant receptor-homologous molecule expressed on T-Helper type 2 cells [CRTH2]) (Nagata *et al.*, 1999). Notably, mast cells, upon IgE-dependent activation, are a main source of PGD2 (Pettipher, Hansel and Armer, 2007). Overall, these findings indicate that the biology of eosinophils may be indirectly influenced by the IgE pathway.

Accumulation of eosinophils at the bronchial level causes damage by degranulation and release of toxic proteins such as eosinophil-derived neurotoxin, eosinophil cationic protein, eosinophil peroxidase, and major basic protein. Airway remodeling is the consequence of ongoing inflammation and repair, and there is increasing evidence that eosinophils are important in the pathophysiology of this process in both allergic and non-allergic asthma. Even though the main, and well-known, function of eosinophils relate to the induction of bronchial wall damage as final effector cells, they also represent the source of a number of regulatory and pro-inflammatory cytokines (IL-3; IL-4; IL-6; GM-CSF; TNF-α; transforming growth factor-β) and chemokines (eotaxins [CCL11]; RANTES [CCL5])(Kita, 2011) .

Effective antigen presentation to Th cells by human eosinophils has been confirmed. In fact, GM-CSF-stimulated human eosinophils can act as antigen-presenting cells to stimulate Th-cell responses against a range of antigens

including allergens, an ability that may help the development of allergic disease (Farhan *et al.*, 2016).

1.2.9. Diagnosis

The diagnosis and severity of asthma are established based on clinical criteria: history, physical examination, and evidence of either reversible airflow obstruction, or airway hyperresponsiveness(Al-Moamary *et al.*, 2016; Hua, Huang and Shen, 2016). The US National Asthma Education and Prevention Program (NAEPP) approach to classifying asthma severity is based on 2 domains: impairment and risk. The impairment domain includes measured airway obstruction, the frequency and intensity of daytime and nocturnal symptoms, frequency of short-acting β2 agonist use for symptom relief, and interference of daily activities by symptoms. The risk domain assesses the frequency of exacerbations. These data collectively define both asthma severity and asthma control. Physical findings of accessory muscle use or audible wheezing during normal breathing may be present only during times of asthma exacerbation and have poor negative predictive value to exclude the diagnosis of asthma. Spirometry is the most important diagnostic procedure for evaluating airway obstruction and its reversibility. It should be performed in all patients in whom asthma is a diagnostic consideration. (Lazarus *et al.*, 2007).

The maximal volume of air forcibly exhaled from the point of maximal inhalation (forced vital capacity, FVC), the volume of air exhaled during the first second of this maneuver (FEV1), and FEV1 :FVC ratio are 3 key measures. An FEV1 :FVC ratio less than the lower limit of normal (0.7-0.8 in adults, depending on age) indicates airway obstruction, although asthma may be present even without demonstrable airway obstruction. Reversibility of airway obstruction is indicated by an increase in FEV1 of 200 mL or greater and 12% or greater from baseline after inhalation of short-acting β2-agonists. In patients who have smoked

cigarettes, distinguishing asthma with partially reversible obstruction from chronic obstructive pulmonary disease is challenging and has led to the description of an asthma– chronic obstructive pulmonary disease overlap syndrome, the existence and clinical importance of which is controversial. No validated approaches for differentiating these entities have been identified. A low diffusing capacity for carbon monoxide suggests an element of emphysema rather than asthma. Pulmonary function testing is less informative when performed during exacerbations of asthma and is best obtained during times of disease stability(Cazzola and Rogliani, 2016).

A total serum IgE and specific IgE for common aeroallergens may be performed, as these tests can guide allergen avoidance strategies and suggest the potential use of anti-IgE monoclonal therapeutics. Allergy skin testing may be substituted for serum measures of allergen- specific IgE. A complete blood cell count with an elevated absolute eosinophil count confident foranti-IL-5 therapies (mepolizumab 150/μL and reslizumab 400/μL). The NAEPP(Al-Moamary *et al.*, 2016) presents a severity classification system based on historical features and spirometric measurements, and recently updated Global Initiative for Asthma (GINA) guidelines are also now available(Hua, Huang and Shen, 2016).

1.2.10. Treatment

Phenotypic presentations in children with asthma are varied and might contribute to differential responses to asthma controller therapy (Fitzpatrick *et al.*, 2016);(Pongracic *et al.*, 2016)). The goal of asthma treatment is to obtain clinical control and reduce future risks to the patient(Pijnenburg *et al.*, 2015). Short term targeted treatment can potentially prevent fall asthma exacerbations while limiting therapy exposure(Teach *et al.*, 2015). Mild to moderate asthma often responds to traditional medications, whereas severe asthma can be refractory to inhaled corticosteroids [ICSs], leukotriene receptor antagonists and

long-acting β-agonists(Berry and Busse, 2016). In addition, children with more severe asthma are often unresponsive to current efforts and there remains a need for agents with properties that may achieve control in these individuals(Darveaux and Busse, 2015). A considerable number of asthmatic children remain symptomatic despite treatment with ICSs, resulting in significant morbidity and reduced quality of life (Vogelberg *et al.*, 2015). Some individuals may be at increased risk of asthma exacerbations despite ICS use, due to genetic factors(Dahlin *et al.*, 2015). Genetic variation may partly explain asthma treatment response heterogeneity(Leusink *et al.*, 2016; Zhang *et al.*, 2017). Whereas genetic associations of the response to ICSs during an asthma exacerbation are unknown(Keskin *et al.*, 2016).

1.2.10.1. Role of montelukast in childhood asthma:

Montelukast is a leukotriene receptor antagonist, approved for the treatment of chronic asthma and prophylaxis and the prevention of exercise-induced bronchoconstriction(Badri and Takov, 2019; Sun and Liu, 2019). Further, it's effective on many biological and pathophysiological mechanisms involved in asthma(Marcello and Carlo, 2016). Montelukast is able to modify the pathophysiological mechanisms of asthma and to improve to some extent the clinical and functional manifestations of the disease (Paggiaro and Bacci, 2011a).

Montelukast is a potent and selective blocker of the cysteinyl leukotrienes receptor [CysLT1R] through binds with high affinity to the cysteinyl leukotriene receptor for leukotrienes D4 and E4 (Badri and Takov, 2019). CysLT1R is pro-inflammatory mediators have numerous effects in the lungs, including the decreased activity of respiratory cilia, increased mucus secretion, increased venopermeability, and promotion of eosinophil migration into airway mucosa(Harmanci, 2007).

1.2.10.2. Role of inhaled corticosteroids [ICSs] in childhood asthma:

Inhaled corticosteroids [ICSs] are the most effective controllers of asthma(Barnes, 2010). Current guidelines recommend long-term treatment with ICS because of their superior effectiveness in managing the chronic airway inflammation that characterizes persistent asthma(Spangler, 2012). The high efficacy of ICS has led to their use in milder disease and younger children in the hope that permanent changes in lung function and airway remodelling may be prevented(Price, 2000) The mechanism of corticosteroids in asthma by enhancing the beta-adrenergic response to relieve the muscle spasm. They also act by reversing mucosal oedema, inhibiting the release of LTC4 and LTD4, decreasing vascular permeability by vasoconstriction, reduce the mucus secretion, and inhibit the late phase reaction by inhibiting the inflammatory response and interfering with chemotaxis(Townley and Suliaman, 1987). In addition, the vascular actions of ICSs contribute to controlling clinical symptoms of asthma primarily by influencing airway calibre in the lung periphery and airway hyper reactivity(Horvath and Wanner, 2006).

1.2.11. Role of immunomarkers in asthma

1.2.11.1. Interleukin 15 (IL 15)

Interleukin (IL)-15 is another notable cytokine with regard to asthma. It is a 4α-helix protein that is structurally similar to IL-2, a growth factor and modulator of T lymphocytes and natural killer (NK) cells, whose increased level is observed in bronchoalveolar lavage cells in asthmatic patients, particularly those with steroid-resistant asthma(McInnes and Gracie, 2004; MAHAJAN and Mehta, 2006) . The IL-15 gene is localized on chromosome 4q31(Anderson *et al.*, 1995). IL-15 mRNA expression occurs in numerous cells, including fibroblasts, epithelial cells, monocytes and dendritic cells(MAHAJAN and Mehta, 2006). Unlike IL-2, whose expression is only detected in activated T lymphocytes, IL-

15 mRNA is subject to low-level constitutive expression that increases in response to infections, particularly in macrophages exposed to lipopolysaccharides and mycobacteria(Kennedy and Park, 1996; Doherty, Seder and Sher, 1996). Although IL-15 itself is not downregulated in the presence of IL-4, IL-13 or TGF-β, its mRNA level has been reported to increase following stimulation by IL-10, which also inhibits other monokines(Kennedy and Park, 1996).

IL-15 is a growth and differentiation factor for NK cells(Leclercq *et al.*, 1996). which also affects the proliferation of B lymphocytes and the production of antibodies(Armitage *et al.*, 1995). It has been demonstrated to have pro-inflammatory properties in rheumatoid arthritis and sarcoidosis(Kirman, Vainer and Nielsen, 1998). However, the role of IL-15 in asthma remains unclear. Certain authors claim that while it does not play any role in the inflammatory process in asthma, it influences the course of pulmonary inflammatory diseases in which the inflammation is mediated by Th1 lymphocytes(Muro *et al.*, 2001). The results of other studies indicate that IL-15 inhibits eosinophil apoptosis(Hoontrakoon *et al.*, 2002), induces IL-5 synthesis through Th cell activity(Mori *et al.*, 1996) and suppresses the Th2-dependent response by promoting the Th1 response in asthmatic mice(Ishimitsu *et al.*, 2001). Experiments on cell lines and the examination of bronchoalveolar lavage fluid have revealed that IL-15 expression in macrophages is increased in Rhinovirus infections, and that this mechanism is impaired in asthmatic patients(Laza-Stanca *et al.*, 2011a). Moreover, IL-15 expression measured in induced expectorated matter and sputum cell culture has been shown to increase following the administration of glucocorticoid therapy(Komai-Koma *et al.*, 2000).

1.2.11.2.Interleukin 22 (IL-22)

Interleukin 22 IL-22, originally described as an IL-10-related T cell derived inducible factor (IL-TIF), belongs to the IL-10 cytokine family (Dumoutier, Louahed and Renauld, 2000). In contrast to the IL-17R, expression of IL-22R is limited mainly on nonhematopoietic tissue cells, such as epithelial cells of the digestive and respiratory systems and keratinocytes of the skin(Wolk *et al.*, 2004). In these cells, IL-22 triggers the production of antimicrobial peptides (although less potent compared to IL-17(Guttman-Yassky *et al.*, 2008) and expression of a series of proteins involved in cellular differentiation and survival(Wolk *et al.*, 2010). IL-22 producing Th cells were first considered to be a variant of Th17 cells, but some studies showed that IL-22 can be produced without production of IL-17 by cells defined as Th22 cells(Nograles *et al.*, 2009; Duhen *et al.*, 2009) Th22 cells coexpress CCR6 and the skinhoming receptors CCR4 and CCR10(Trifari *et al.*, 2009). suggesting that these cells have a prominent function in the skin. IL-22 has both pro-inflammatory (again to a lesser extent than IL-17) and protective properties, but the dual effects of IL-22 are not yet fully understood. IL-22 deficient mice have a lower disease incidence in a rheumatoid arthritis model(Geboes *et al.*, 2009) and IL-22 induces keratinocyte migration in an in vitro injury model and strongly induces hyperplasia of reconstituted human epidermis(Boniface *et al.*, 2005). In addition, while IL-22 deficient mice are highly susceptible to liver damage in an experimental hepatitis model, transfer of IL-22-expressing Th17 cells into IL-22- deficient mice results in protection(Zenewicz *et al.*, 2007). In line with increased levels of IL-17 in the lung of severe asthmatics, the percentages of Th17 cells , as well as the plasma concentrations of IL-17 and IL-22, tended to increase with disease severity(Zhao *et al.*, 2010a).It has been reported that IL-22 levels are increased in the sera of asthma patients and are positively correlated with disease severity (Zhao *et al.*, 2010a; Besnard *et al.*, 2011; Zhu *et al.*, 2011). The majority of IL-22-producing

cells in peripheral blood of asthma patients are CD4+ CCR6+ CD161+ cells(Cosmi *et al.*, 2010), suggesting that Th17 cells are the main producer of IL-22. In contrast, it has been reported that the majority of IL-22-producing CD4+ T cell lines which are generated from lung biopsy specimens of asthma patients produce IFN-γ. (Pennino *et al.*, 2013).

Regarding the function of IL-22 in asthma patients, Pennino *et al.* have showed that IL-22 inhibits IFN-γ-induced expression of proinflammatory chemokines and adhesion molecules in human bronchial epithelial cells. They have also shown that the levels of IL-22 in the BALF of asthma patients are inversely correlated with the levels of proinflammatory chemokines, suggesting the protective roles of IL-22 in asthma patients(Pennino *et al.*, 2013). On the other hand, it has been shown that IL-22R1 is expressed on not only lung epithelial cells but also airway smooth muscle cells (ASMCs) in humans and that IL-22 enhances the proliferation and migration of human ASMCs], suggesting that IL-22 may involve in smooth muscle cell hyperplasia, a key pathological feature of asthma, in human airways. Taken together, these studies suggest that IL-22 plays inhibitory roles in the development of allergic airway inflammation in asthma patients, but it could promote airway remodeling if its expression is uncontrolled during the resolution phase of allergic inflammation.(Chang *et al.*, 2011; Chang *et al.*, 2012; Kudo *et al.*, 2012).

1.2.11.3. Interleukin 23 (IL-23)

Interleukin-23(IL-23) is a hetero-dimeric cytokine which is consisted of a P19 (IL-23α chain) a subunit specific for IL-23 and the p40 subunit of IL-12. P19 is specific, however P40 chain also exists in the structure of IL-12 and is a shared subunit between the two interleukins (Oppmann *et al.*, 2000). IL-23 receptor (IL-23R) is expressed on T cells (especially Th17), natural killer cells, monocytes and dendritic cells (McGeachy *et al.*, 2009). This hetero-dimeric receptor is made up

from two parts including IL-12RB1 (shared between IL-23 and IL-12) and another receptor known as IL-23Rα which is specific for IL-23 (McGeachy *et al.*, 2009; Floss *et al.*, 2015). Activated macrophages and dendritic cells of peripheral blood produce a significant amount of IL-23 (Oppmann *et al.*, 2000). The most commonly presumed function of IL-23 is to expand differentiated Th17 cells or maintain IL-17 production (McGeachy and Cua, 2008). IL-23 may function on dendritic cells to modulate their activity on Th2 cell differentiation; Therefore, IL-23 not only differentiates Th17 cells but also helps in Th2 cell differentiation (Wakashin *et al.*, 2008). IL-23 has a substantial role in causing eosinophilic inflammation in the airways; however, still few studies have been conducted on IL-23 serum levels and its association with in pediatric asthma (Ciprandi *et al.*, 2012b).

Interleukin-23 is crucial for the maintenance of Th17 cell(Langrish *et al.*, 2005) and it is required for full acquisition of an effector function of Th17 cells(McGeachy *et al.*, 2007). Furthermore, IL-23 prolongs the expression of Th17 cytokines, such as IL-17A, IL-17F, IL-22, and GM-CSF that induce tissue pathology and chronic inflammatory diseases(Gaffen *et al.*, 2014). These effects identify IL-23 as a key cytokine in the Th17 cells/IL-17 inflammatory axis in the pathogenesis of many autoimmune and chronic inflammatory diseases(Duvallet *et al.*, 2011).

IL-23 is implicated in several chronic inflammatory diseases, such as rheumatoid arthritis(Chabaud *et al.*, 1999; Robert and Miossec, 2018). Psoriasis (Di Cesare, Di Meglio and Nestle, 2009; Nakajima, 2012). inflammatory bowel disease (Mannon *et al.*, 2004; Sarra *et al.*, 2010), and neutrophilic asthma(Nakajima and Hirose, 2010; Syabbalo, 2020a).

Ciprandi and colleagues (2012) have found that serum IL-23 levels were increased in allergic asthmatic children not on corticosteroids treatment,

compared with non-allergic children, and IL-23 levels were strongly and inversely correlated with lung function (FEV1), and airflow limitation in small airways (FEF25-75%). Interleukin-23 can be used as a biomarker of airflow obstruction in patients with neutrophilic asthma(Ciprandi, Cuppari and Salpietro, 2012).

Chapter Two
Materials and Methods

2. Material and Methods:

2.1. Subjects and Study Design:

2.1.1. Subjects:

This study included consecutive asthmatic children attending the asthma clinic at Karbala Teaching Hospital for Children in the period extending from November 2020 to March 2021. All asthmatic children were clinically diagnosed according to the American Thoracic Society criteria for asthma (Chung and Wenzel, 2014). This study involved 60 asthmatic children (36 male and 24 female) and 60 non-asthmatic children (32 male and 28 female). The non-asthmatic children (control) were matched in age and gender with asthmatic children. Information of Case sheets involving age, sex, family history, duration and others were carried out for each participant (appendix I asthmatic patients' and controls questionnaires).

2.1.2. Inclusion and Exclusion Criteria:

2.1.2.1. **Inclusion criteria:** Children were diagnosed with asthma at the asthma clinic of Karbala Teaching Hospital for Children either newly diagnosed or on controller therapy.

2.1.2.2. **Exclusion criteria:** Autoimmune disease (rheumatoid arthritis, inflammatory bowel disease and others), tumour and COPD.

2.1.3. Study Design:

This study is a cross-sectional study

2.1.3.1. Flow chart

A flow chart that was enrolled in this study was shown in (figure 2.1)

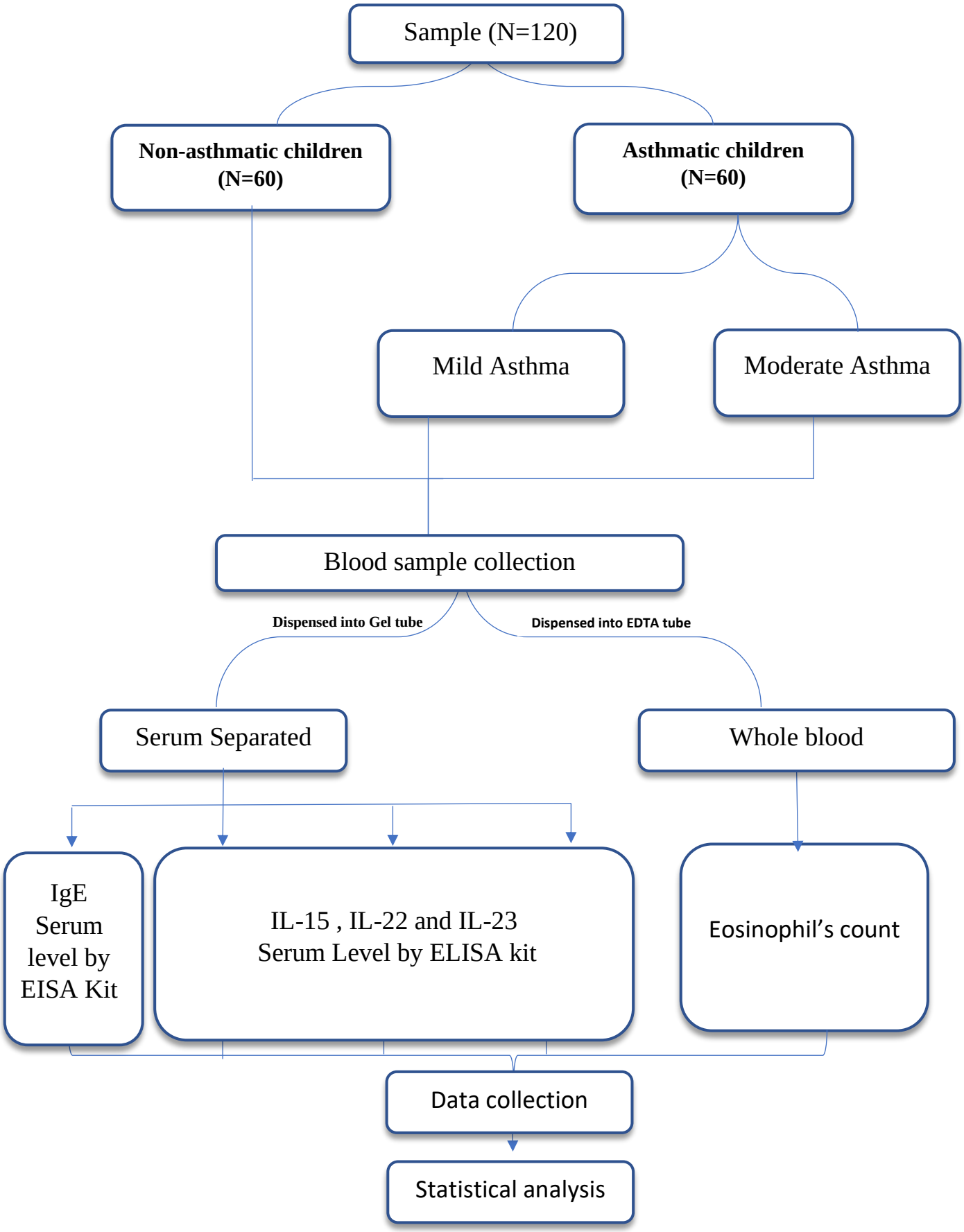

Figure 2.1: A flow chart illustrating the study design

2.2. Ethical Approval:

Ethic's approval was obtained from Karbala Health Directorate. In addition, verbal consent was taken from their parents since patients were minors before taking the sample. Health measures and safety were taken when sampling.

2.3. Materials:

2.3.1. Equipment and Instruments:

In the present study, the following Equipment and Instruments were used (table 2-1).

Table 2-1: Equipment and Instruments

Equipment and Instruments	Manufacturing Company	Country
Bench centrifuge	Hettich	Germany
BioTek ELx800 automated immunoassay analyzer	BioTek	USA
EDTA tube	Al Rawan	China
ELISA instrument system	Human	Germany
Eppendorf tube (0.5 ml & 1.5 ml)		China
Freezer	Panasonic	Korea
Gel Tube	Al Rawan	China
Gloves	Top glove SDN. BHD	Malaysia
Hematology analyzer	Sysmex XN 350	Japan

Micropipette tips	BIOBASIC	Canada
Micropipettes	BIOBASIC	Canada
Microtiter Plate Reader	BioTek	USA
Microtiter Plate Washer	BioTek	USA

2.3.2. ELISA Kit:

ELISA kits used in the present study are illustrated in (table 2-2)

Table 2-2: ELISA Kits used in the study

Elisa Kit	Manufacturing Company	Country
Human IL-15(Interleukin 15) ELISA Kit Catalog No : E-EL-H0222	Elabscience	China
Human IL-22(Interleukin 22) ELISA Kit Catalog No : E-EL-H0106	Elabscience	China
Human IL-23(Interleukin 23) ELISA Kit Catalog No : E-EL-H0107	Elabscience	China
Total serum IgE Catalog No : DEIGE02	Demeditec Diagnostics GmbH	Germany

2.5.2.1. ELISA Kit Content of Total Serum IgE:

The content of the total serum IgE that contain component and format were shown in table (2-3).

Table 2-3: ELISA kit for detection of human total serum IgE components.

Components	Format
Microplate wells coated with mouse monoclonal anti-IgE	12×8
Calibrators (Standards) (0, 5, 25, 100, 250, 1000 IU/mL)	1x 1 ml; 5x 200 µl
Enzyme Conjugate (goat anti-human-IgE-HRP, in protein-containing buffer solution)	22 ml
Substrate: TMB (tetramethylbenzidin).	12 ml
Stop Solution 1 N acidic solution	12 ml
Washing Buffer PBS + Tween 20, 10x concentrate. dilute1+9 with distilled water	60 ml

2.5.2.2. Human IL-15 (Interleukin 15) ELISA Kit

The content of the serum IL-15 that contain component and format were shown in table (2-4).

Table 2-4: Human IL-15(Interleukin 15) ELISA Kit for detection of human total serum IL-15 components.

Components	Format
Micro ELISA Plate	12×8

Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1×120 µl
Concentrated HRP Conjugate (100×)	1×120 µl
Reference Standard & Sample Diluent	1 × 20 ml
Biotinylated Detection Ab Diluent	1 × 14 ml
HRP Conjugate Diluent	1×14 ml
Concentrated Wash Buffer (25×)	1×30 ml
Substrate Reagent	1× 10 ml
Stop Solution	1× 10 ml
Plate Sealer	5 pieces

2.5.2.3. Human IL-22(Interleukin 22) ELISA Kit

The content of the serum IL-22 that contain component and format were shown in table (2-5).

Table 2-5: Human IL-22(Interleukin 22) ELISA Kit components.

Components	Format
Micro ELISA Plate	12×8
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1×120 µl

Concentrated HRP Conjugate (100×)	1×120 µl
Reference Standard & Sample Diluent	1 × 20 ml
Biotinylated Detection Ab Diluent	1 × 14 ml
HRP Conjugate Diluent	1×14 ml
Concentrated Wash Buffer (25×)	1×30 ml
Substrate Reagent	1× 10 ml
Stop Solution	1× 10 ml
Plate Sealer	5 pieces

2.5.2.4. Human IL-23(Interleukin 23) ELISA Kit

The content of the serum IL-23 that contain component and format were shown in table (2-6).

Table 2-6: Human IL-23(Interleukin 23) ELISA Kit component

Components	Format
Micro ELISA Plate	12×8
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1×120 µl
Concentrated HRP Conjugate (100×)	1×120 µl

Reference Standard & Sample Diluent	1 × 20 ml
Biotinylated Detection Ab Diluent	1 × 14 ml
HRP Conjugate Diluent	1×14 ml
Concentrated Wash Buffer (25×)	1×30 ml
Substrate Reagent	1× 10 ml
Stop Solution	1× 10 mlrf
Plate Sealer	5 pieces

2.6. Methods:

2.6.1. Sample Collection:

Approximately 5 ml of venous blood were drawn from each participant 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 was dispensed into EDTA tube for the hematological test. Four ml of blood was dispensed into Gel tube and allowed to clot then serum was separated by centrifugation at 3000 round per minutes (RPM) for 10 minutes. Then the serum was transferred into Four prelabeled Eppendorf tubes by micro-pipette and stored at -20 C˚ for subsequent immunological assays.

2.6.2. 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 hematology analyzer (Sysmex, Japan) to count Eosinophils.

2.6.3. ELISA Procedures

2.6.3.1. Measurement of Total Serum IgE

Serum was analysed to determine the total IgE concentration by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Demeditec Diagnostics GmbH Total serum IgE (Catalog No : DEIGE02).

2.6.3.1.1. The Principle of The Test:

The test kit contains microtiter wells surface coated with A monoclonal mouse-anti-human IgE antibody Undiluted patient serum or ready-to-use standards are 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 color development was terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye was measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgE antibodies was directly proportional to the intensity of the color.

2.6.3.1.2. Procedure of The Test:

1. A sufficient amount of microtiter wells for the standards and samples in duplicate as well as for a substrate blank were prepared.
2. A 10 µL each of the undiluted samples and the ready-to-use standards together with 200 µL of conjugate were pipetted into the wells. leaving one well empty for the substrate blank.
3. Then the plate was covered with the re-usable plate cover and incubate at room temperature for 30 minutes.

4. Then the wells of the plate were Emptied (dump or aspirate) and added 300 µL of diluted washing solution. This procedure was repeated totally three times. the Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. A100 µL each of the ready-to-use substrate as well as the substrate blanks were pipetted into the wells.
6. Then the plate was covered with the re-usable plate cover and incubate at room temperature for 15 minutes in the dark (e.g. drawer)
7. Then 100 µL of the ready-to-use stop solution were pipetted into the wells To terminate the substrate as well as the substrate blank reaction.
8. After thorough mixing and wiping the bottom of the plate the reading of the absorption at 450 nm was performed.

2.6.3.2. Measurement of Total Serum IL-15:

Total IL-5 concentration was measured by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Elabscience Human IL-15(Interleukin 15) ELISA Kit Catalog No : E-EL-H0222).

2.6.3.2.1. The Principle of The Test:

This test uses the Sandwich-ELISA principle,The micro ELISA plate has been pre-coated with an antibody specific to Human IL-15. Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-15 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution then added to each well. Only those wells that contain Human IL-15, biotinylated detection antibody and Avidin-HRP conjugate had appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured

spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value then was proportional to the concentration of Human IL-15. We calculated the concentration of Human IL-15 in the samples by comparing the OD of the samples to the standard curve (Appendix II).

2.6.3.2.2. Procedure of The Test:

1. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (100 uL for each well). Then the samples were added to the other wells (100 uL for each well). Later the plate was covered with the sealer provided in the kit. And Incubated for 90 min at 37
2. The liquid out of each well was removed, without washing. Immediately 100 µL of Biotinylated Detection Ab working solution was added to each well. Covered with the Plate sealer. Gently mixed up. Incubated for 1 hour at 37°C.
3. After the solution from each well was decanted, 350 uL of wash buffer was added to each well. Soaked for 1~2 min and the solution from each well decanted and pat it to dried against clean absorbent paper. this wash step was repeated 3 times.
4. Then 100 µL of HRP Conjugate working solution was added to each well. Covered with the Plate sealer. And incubated for 30 min at 37°C.
5. Later the solution from each well was decanted, And the wash process was repeated five times as conducted in step 3.
6. A 90 µL of Substrate Reagent was added to each well. Covered with a new plate sealer. Incubated for about 15 min at 37°C.
7. Later 50 µL of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.6.3.3. Measurement of Total Serum IL-22

Serum was analyzed to determine the total IL-22 concentration by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Elabscience Human IL-22(Interleukin 22) ELISA Kit Catalog No : E-EL-H0106.

2.6.3.3.1. Principle of Test:

This test uses the Sandwich-ELISA principle , The micro ELISA plate has been pre-coated with an antibody specific to Human IL-22. Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-22 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution then added to each well. Only those wells that contain Human IL-22, biotinylated detection antibody and Avidin-HRP conjugate had appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value then was proportional to the concentration of Human IL-22. We calculated the concentration of Human IL-22 in the samples by comparing the OD of the samples to the standard curve (Appendix II).

2.6.3.3.2. Procedure of The Test

1. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (100 uL for each well). Then the samples were added to the other wells (100 uL for each well). Later the plate was covered with the sealer provided in the kit. And Incubated for 90 min at 37

2. The liquid out of each well was removed, without washing. Immediately 100 μL of Biotinylated Detection Ab working solution was added to each well. Covered with the Plate sealer. Gently mixed up. Incubated for 1 hour at 37°C.
3. After the solution from each well was decanted, 350 uL of wash buffer was added to each well. Soaked for 1~2 min and the solution from each well decanted and pat it to dried against clean absorbent paper. this wash step was repeated 3 times.
4. Then 100 μL of HRP Conjugate working solution was added to each well. Covered with the Plate sealer. And Incubated for 30 min at 37°C.
5. Later the solution from each well was decanted, And the wash process was repeated five times as conducted in step 3.
6. A 90 μL of Substrate Reagent was added to each well. Covered with a new plate sealer. Incubated for about 15 min at 37°C.
7. Later 50 μL of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.6.3.4. Measurement of Total Serum IL-23

Serum was analyzed to determine the total IL-23 concentration by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Elabscience Human IL-23(Interleukin 23) ELISA Kit Catalog No : E-EL-H0107.

2.6.3.4.1. Principle of Test:

This test uses the Sandwich-ELISA principle , The micro ELISA plate has been pre-coated with an antibody specific to Human IL-23. Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-23 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to

each micro plate well and incubated. Free components were washed away. The substrate solution then added to each well. Only those wells that contain Human IL-23, biotinylated detection antibody and Avidin-HRP conjugate had appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value then was proportional to the concentration of Human IL-23. We calculated the concentration of Human IL-23 in the samples by comparing the OD of the samples to the standard curve(appendix II)

2.6.3.4.2. Procedure of The Test

1. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (100 uL for each well). Then the samples were added to the other wells (100 uL for each well). Later the plate was covered with the sealer provided in the kit. And Incubated for 90 min at 37
2. The liquid out of each well was removed, without washing. Immediately 100 μL of Biotinylated Detection Ab working solution was added to each well. Covered with the Plate sealer. Gently mixed up. Incubated for 1 hour at 37°C.
3. After the solution from each well was decanted, 350 uL of wash buffer was added to each well. Soaked for 1~2 min and the solution from each well decanted and pat it to dried against clean absorbent paper. this wash step was repeated 3 times.
4. Then 100 μL of HRP Conjugate working solution was added to each well. Covered with the Plate sealer. And Incubated for 30 min at 37°C.
5. Later the solution from each well was decanted, And the wash process was repeated five times as conducted in step 3.

6. A 90 µL of Substrate Reagent was added to each well. Covered with a new plate sealer. Incubated for about 15 min at 37°C.
7. Later 50 µL of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.7. Statistical analysis:

Data was introduced into a Specific Software Statistical Package for the Social Sciences (SPSS) version 22 for windows (San Diego, California, USA) to do statistical analysis, while the figures constructed was by EXEL program of Microsoft Office 2019 (GraphPad prism Microsoft).

The results were expressed as mean ± SD. Comparisons between two means were performed using T test, while ANOVA was using to compare among mean. A p value of <0.05 was considered to indicate the statistical significance and highly significant if p-value <0.001. Chi-square (x^2) used to compare between two categorical variables. In addition, the pearson correlation was used to explain the relation between IL-15, IL-22 and IL-23 levels with absolute eosinophil count and with total serum IgE levels.

Chapter Three
Results

3. Results:

3.1. Demographical Distribution of Asthmatic Patients and Control:

Asthmatic patient's ages ranged between 4 months to 14 years old; there were no significant differences in age distribution between asthmatic patients and control, the means of ages are (7.89 ±4.29) and (8.50 ± 3.88) for asthmatic children and control respectively (*P =0.413*), as shown in table (3-1).

The study result revealed a higher distribution of asthma in males than in females, with a percentage of 35(58.3%) and 25(41.7%), respectively. There was no significant difference (*P = 0.317*) in gender distribution between the patients and control.

The present study found that 2(3.3%) and 1 (1.7%) of patients and control respectively had a positive result for the history of eczema, while 58(96.7%) and 59(98.3%) of those have a negative result. There was no significant difference (*P = 0.679*) in the history of eczema distribution between the patients and control.

Regarding the family history of eczema, found 7(11.7%) and 0 (0.0%) of patients and healthy control respectively had a positive family history of eczema while 53 (88.3%) and 60 (100%) of those have a negative result. There was a significant difference (*P = 0.013*) between the patients and the control family history of eczema.

The current study found that 11(18.3%) and 12 (20%) of patients and control respectively had a positive allergic rhinitis, while 49 (81.7%) and 48(80%) of those had a negative result. There was no significant difference (*P = 0.817)* in allergic rhinitis distribution between the patients and control.

Concerning the family history of allergic rhinitis, found 30(50%) and 8(13.3%) of patients and control respectively had a positive result while 30(50%) and 52 (86.7%) of those have a negative result. There was a significant difference

(*P = 0.005*) in the family history of allergic rhinitis distribution between the patients and control.

This study found that 17(28.3%) and 8 (13.3%) of patients and control respectively had a positive allergic conjunctivitis, while 43(71.7%) and 52(867%) of those had a negative result. There was no significant difference (*P = 0.071*) in the distribution of allergic conjunctivitis between the patients and the control.

The current study found that 30 (50%) and 12 (20%) of patients and control, respectively had a positive result for exposure to cigarette smoke while 30 (50%) and 48(80%) of those had a negative result. There was a highly significant difference (*P = 0.001*) in exposure to cigarette smoke distribution between the patients and control.

Table 3-1: Distribution of demographic characteristics for patients and control

Variables		**Group**				***P-value***
		Patients N=60		**Control N=60**		
		Mean	SD	Mean	SD	
Age		7.89	4.29	8.50	3.88	0.413
		Count	%	Count	%	
Gender	Male	35	58.3%	32	53.3%	0.317
	Female	25	41.7%	28	46.7%	
History of Eczema	+ve	2	3.3%	1	1.7%	0.679
	-ve	58	96.7%	59	98.3%	
Family History of eczema	+ve	7	11.7%	0	0.0%	**0.013***
	-ve	53	88.3%	60	100.0%	
Allergic rhinitis	+ve	11	18.3%	12	20.0%	0.817
	-ve	49	81.7%	48	80.0%	
Family History of allergic rhinitis	+ve	30	50.0%	8	13.3%	**0.005***
	-ve	30	50.0%	52	86.7%	
Allergic conjunctivitis	+ve	17	28.3%	8	13.3%	0.071
	-ve	43	71.7%	52	86.7%	
Exposure to cigarette smoke	+ve	30	50.0%	12	20.0%	**0.001****
	-ve	30	50.0%	48	80.0%	

**** p-value* is significant*(P<0.05)*, ** *p-value* is highly significant, Student's t-test, Chi-square test**

3.2. Mean differences serum levels of immunological markers:

The current study showed that the (mean ± SE) of eosinophils count were (0.40 ± 0.06) and (2.14 ± 0.24) in patients and control respectively there was a significant difference between patients and control in eosinophil counts , it was higher in control (*p =0.005*), as shown in table (3-2).

Also, this study found that the IgE serum level were (126.21 ±14.57) and (37.65 ±5.14) in patients and control respectively. There was a significant difference between patients and control in IgE serum level (*p =0.005*).

Regarding the serum level of IL-15 were (39.69 ±8.91) and (39.15 ±9.89) in patients and control, respectively, there was no significant difference between patients and control in IL-15 serum level (*p = 0.968*).

In addition, the current study found that serum levels of IL-22 were (41.54 ±10.05) and (36.57 ±9.55) in patients and control, respectively. There was no significant difference between patients and control in IL-22 serum level (*p = 0.721*).

In current study found that serum levels of IL-23 were (54.12 ±17.81) and (5.12 ±2.50) in patients and control respectively. There was a significant difference between patients and control regarding IL-23 serum levels (*p-value = 0.008*), as clarified in table (3-2).

Table 3-2: Mean differences of IL-15, IL-22 and IL-23 serum level among the patients and control

Variables	Group				*P-value*
	Patients		Control		
	Mean	SE	Mean	SE	
EOS (cell/mm^3)	0.40	0.06	2.14	0.24	**0.005***
IgE (pg/ml)	126.21	14.57	37.65	5.14	**0.005***
IL 15 (pg/ml)	39.69	8.91	39.15	9.89	0.968
IL 22 (pg/ml)	41.54	10.05	36.57	9.55	0.721
IL 23 (pg/ml)	54.12	17.81	5.12	2.50	**0.008***

*** *p-value* is significant *(P < 0.05)*, Student's t-test, EOS=Eosiniphil, IgE=Immunoglobulin E, IL=Interleukin**

3.3. The relation of aggravating factors and their exposure with the severity in asthmatic patients:

This study (4.3%) of moderate patients were positive for direct contact with cats. In comparison (100 %) and (95.7%) of both mild and moderate asthmatic patients had negative for direct contact with cats, there were no significant differences between mild and moderate asthma (*P-value = 0.427*).

Also, in the present study (4.3%) of moderate asthma patients were positive for direct contact with dogs while (100%) and (95.7%) of both mild and moderate

asthmatic patients had negative for direct contact with dogs, there were no significant differences between mild and moderate asthma (*P-value = 0.585*).

In addition, 14.3 % and 10.9% of both mild and moderate asthma patients respectively were positive for direct contact to birds while 85.7% and 89.1% of both mild and moderate asthmatic patients respectively had negative for direct contact with birds, there were no significant differences between mild and moderate asthma (*P-value = 0.727)*.

The study also showed that 64.3 and 30.4 of both mild and moderate asthmatic patients respectively were highly aggravating by upper respiratory inflammation, while 35.7% and 69.6% of both mild and moderate asthmatic patients respectively were not, there were a significant differences between mild and moderate asthma regarding aggravating by upper respiratory inflammation (*P-value = 0.031*).

Regarding aggravated by dust, the study showed that 35.7% and 32.6% of both mild and moderate asthmatic patients respectively are positive while 64.3% and 67.4% of both mild and moderate asthmatic patients respectively were a negative result, there was no significant difference between the severity of asthma and aggravating by dust (*P-value = 0.535*).

Also, this study showed that 64.3% and 50% of both mild and moderate asthmatic patients respectively are aggravated by playing or exercising, while 35.7% and 50% of both mild and moderate asthmatic patients respectively were not, there was no significant difference between the severity of asthma and aggravating by physical activities (*P-value = 0.379*).

Concerning treatment, 57.1% and 19.6% of the mild and moderate asthmatic patients respectively were under no treatment. 35.7% and 56.5% of which were taking Montelukast as primary treatment, while 7.1 % and 19.6% took inhaled corticosteroids (ICS) as the primary treatment. Only 4.3 of moderate

were under mixed treatment of ICS and Montelukast. There was a significant difference regarding the treatment between mild and moderate asthmatic patients (*P-value = 0.049*).

Also, in this study 42.9 % and 45.7% of both mild and moderate asthmatic patients respectively were diagnosed as early wheezier, while 7.1% and 6.5% of which were diagnosed as allergic asthma. 7.1% and 6.5% of which were diagnosed as non-allergic asthma. 14.3% and 13% were adolescent (obese) asthma, 14.3% and 8.7% of both mild and moderate asthmatic patients respectively were diagnosed as late-onset asthma while 14.3% and 19.6% of both mild and moderate asthmatic patients respectively were diagnosed as persisting wheezier. There was a non-significant difference regarding the asthma phenotype between mild and moderate asthmatic patients (*P = 0.991*), as demonstrated in table (3-3).

Table 3-3: The relation of severity with aggravating factors, treatment and phenotypes in asthmatic patients

Variables			Severity				*P-value*
			Mild		Moderate		
			Count	%	Count	%	
Pets	Cats	+ve	0	0.0%	2	4.3%	0.427
		-ve	14	100.0%	44	95.7%	
	Dogs	+ve	0	0.0%	2	4.3%	0.585
		-ve	14	100.0%	44	95.7%	
	Birds	+ve	2	14.3%	5	10.9%	0.727
		-ve	12	85.7%	41	89.1%	

Aggravating by upper respiratory inflammation	+ve	9	64.3%	14	30.4%	**0.031***
	-ve	5	35.7%	32	69.6%	
Aggravating by dust	+ve	5	35.7%	15	32.6%	0.535
	-ve	9	64.3%	31	67.4%	
Aggravating by physical activities	+ve	9	64.3%	23	50.0%	0.379
	-ve	5	35.7%	23	50.0%	
Treatment	No treatment	8	57.1%	9	19.6%	**0.049***
	Montelukast	5	35.7%	26	56.5%	
	ICS	1	7.1%	9	19.6%	
	Mixed	0	0.0%	2	4.3%	
Asthma phenotypes	Early wheeze	6	42.9%	21	45.7%	0.991
	Allergic asthma	1	7.1%	3	6.5%	
	Non-allergic	1	7.1%	3	6.5%	
	Adolescent & obesity	2	14.3%	6	13.0%	
	Late-onset	2	14.3%	4	8.7%	
	Persist wheezier	2	14.3%	9	19.6%	

*** *p-value* is significant*(P<0.05)*, Chi-square test, Inhaled Corticosteroids (ICS)**

3.4. Correlation between the studied markers

The correlation between the studied markers are clarified in table (3-4) and the figures (3-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17).

Table 3-4: Correlation of the studied markers in patients

Variables	Correlations	EOS	IgE	IL 15	IL 22	IL 23	Duration
EOS	r		.051	.112	.017	.064	-.061
	P-value		.697	.395	.900	.625	.646
	N		60	60	60	60	60
IgE	r	.051		-.136	-.040	-.170	-.017
	P-value	.697		.301	.762	.194	.898
	N	60		60	60	60	60
IL 15	r	.112	-.136		.215	.066	.055
	P value	.395	.301		.099	.618	.675
	N	60	60		60	60	60
IL 22	r	.017	-.040	.215		.081	.038
	P value	.900	.762	.099		.538	.772
	N	60	60	60		60	60
IL 23	r	.064	-.170	.066	.081		**-.280***
	P value	.625	.194	.618	.538		**.030**
	N	60	60	60	60		**60**
Duration	r	-.061	-.017	.055	.038	**-.280***	
	P value	.646	.898	.675	.772	**.030**	
	N	60	60	60	60	**60**	

***. Correlation is significant at the 0.05 level (2-tailed).**

N: patients number, r: correlation coefficient

3.4.1. Correlation of Eosinophils, IgE and immunological markers with the duration in asthmatic patients as shown in table (3-4)

3.4.1.1. Correlation between Eosinophils count and the duration of asthma

Figure (3-1) showed the correlation between EOS count and disease duration in months for asthmatic patients. No significant negative correlation were found between EOS count and duration of disease, r= -0.61 (*p = 0.646*).

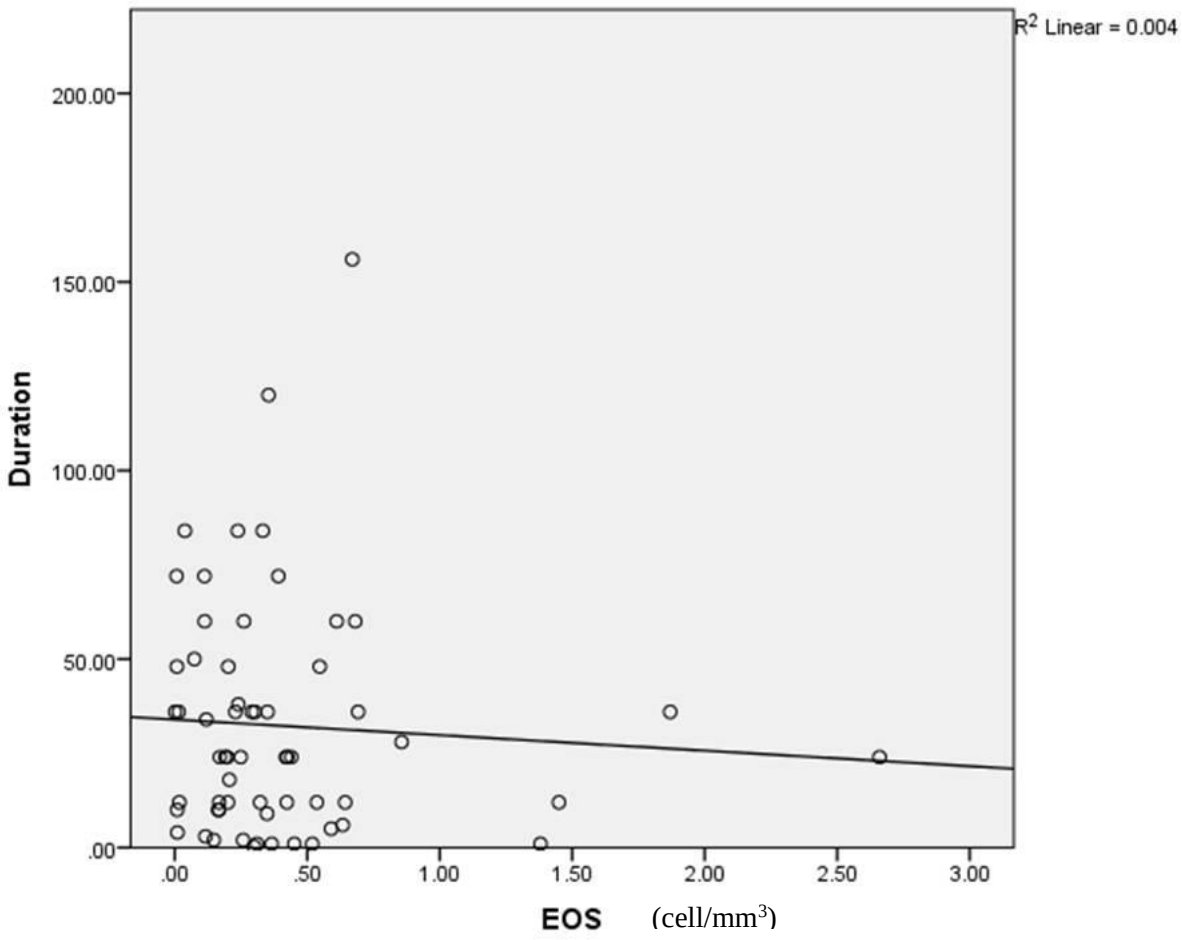

Figure (3-1): Correlation of eosinophil count with the duration of asthma in months. Pearson correlation coefficient: r= -0.61 (*p = 0.646*)

3.4.1.2. Correlation between IgE serum level and duration of asthma

Figure (3-2) showed the correlation between IgE and disease duration in months of asthmatic patients. No significant negative correlation were found between IgE serum level and duration of disease, r= -0.017 (*p = 0.898*).

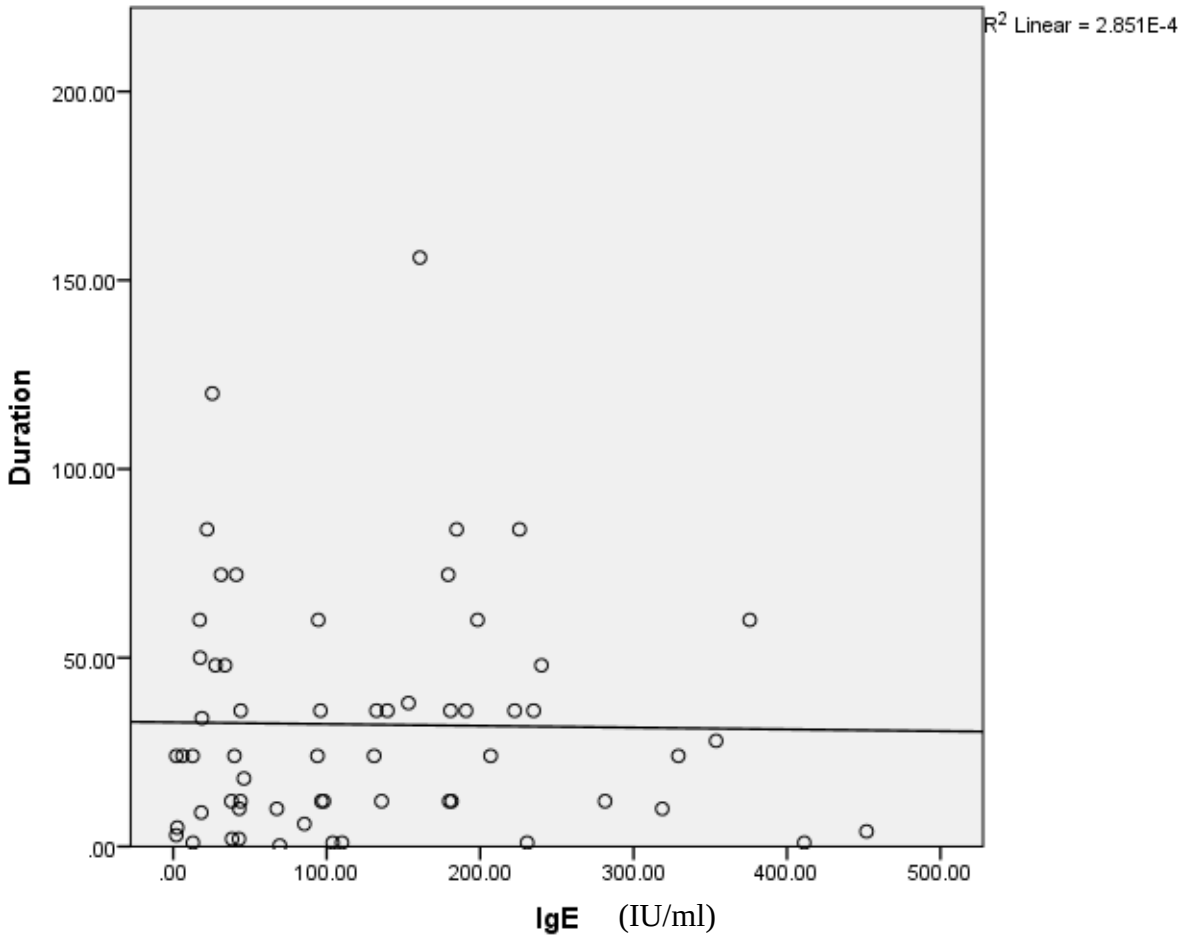

Figure (3-2): Correlation of IgE serum level with the duration of asthma in months. Pearson correlation coefficient: r= -0.017 (*p = 0.898*).

3.4.1.3. Correlation between serum interleukin 15 (IL-15) level and duration of asthma

Figure (3-3) showed the correlation between IL-15 serum level and duration of disease in months of asthmatic patients. No significant correlation were found between IL-15 serum level and duration of disease, r= 0.055 (*p = 0.675*).

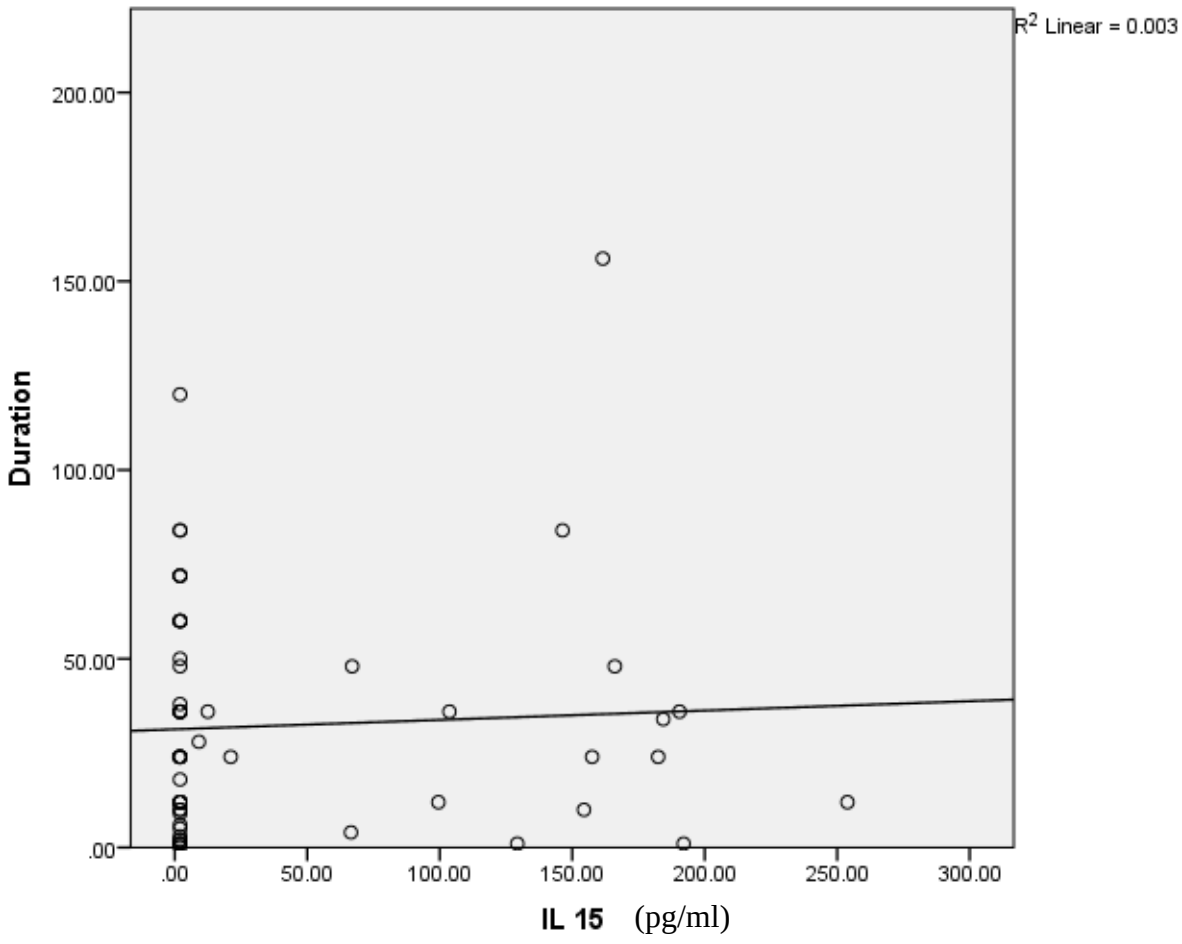

Figure (3-3): Correlation of IL-15 serum level with the duration of asthma in months. Pearson correlation coefficient: r= 0.055 (*p = 0.675*).

3.4.1.4. Correlation between serum interleukin 22 (IL-22) level and duration of asthma

Figure (3-4) showed the correlation between IL-22 and duration of disease in months of asthmatic patients. No significant correlation were found between IL-22 serum level and duration of disease, r= 0.38 (*p = 0.772*).

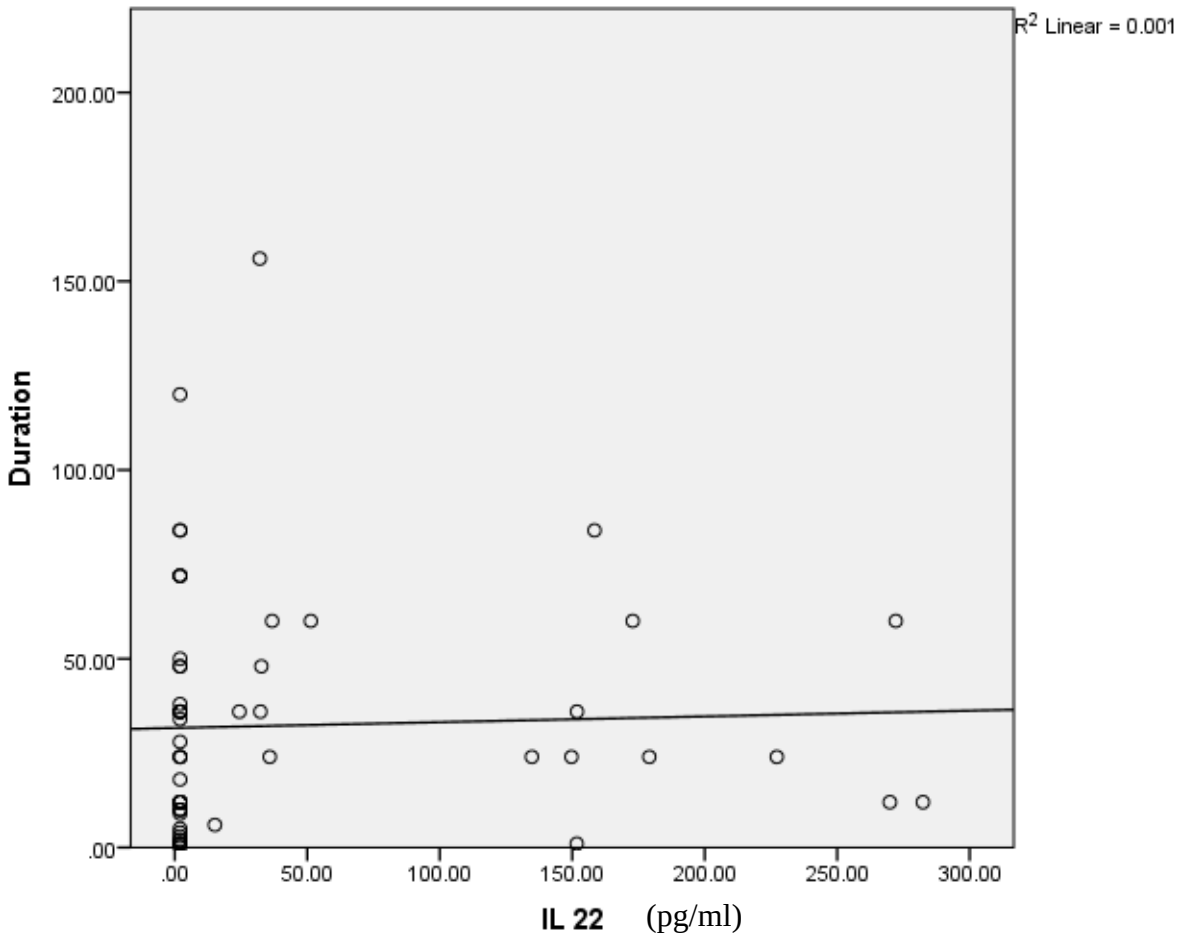

Figure (3-4): Correlation of IL-22 serum level with the duration of asthma in months. Pearson correlation coefficient: r= 0.38 (*p = 0.772*).

3.4.1.5. Correlation between serum interleukin 23 (IL-23) level and duration of asthma

Figure (3-5) showed the correlation between IL-23 and duration of disease in months of asthmatic patients. There were a significant negative correlation between IL-23 level and duration of asthma r= - 0.280, (*p = 0.030*).

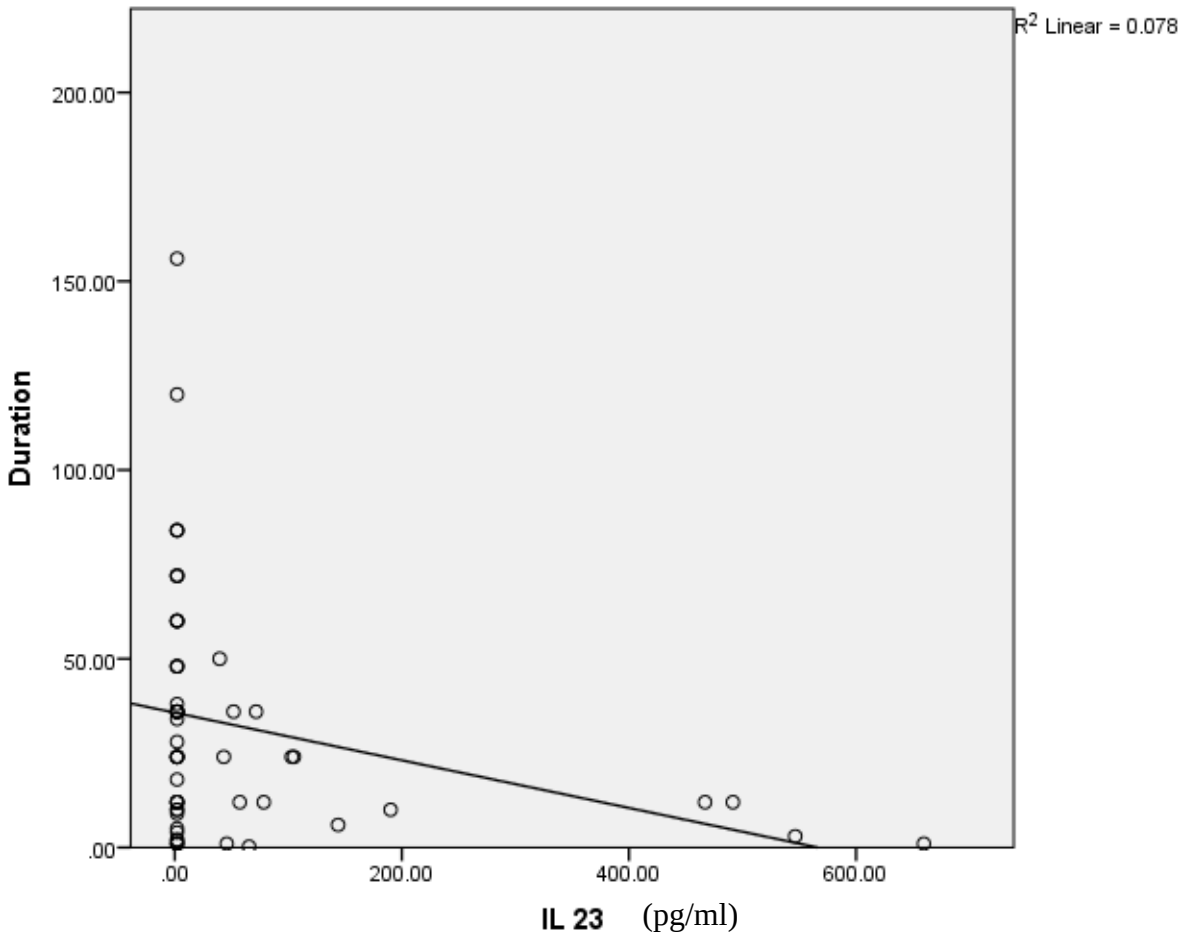

Figure (3-5): Correlation of IL-23 serum level with the duration of asthma in months. Pearson correlation coefficient: r= -0.280 (*p = 0.30*).

3.4.2. Correlation of IL-15 serum level and immunological parameters

3.4.2.1. The Correlation between IL-15 serum Level and Eosinophil Count in asthmatic patients

Figures (3-6) showed the correlation between the serum IL-15 level and the eosinophil count. No significant positive correlation were found between IL-15 serum level and eosinophil count, r= +0.112 (*p = 0.395*).

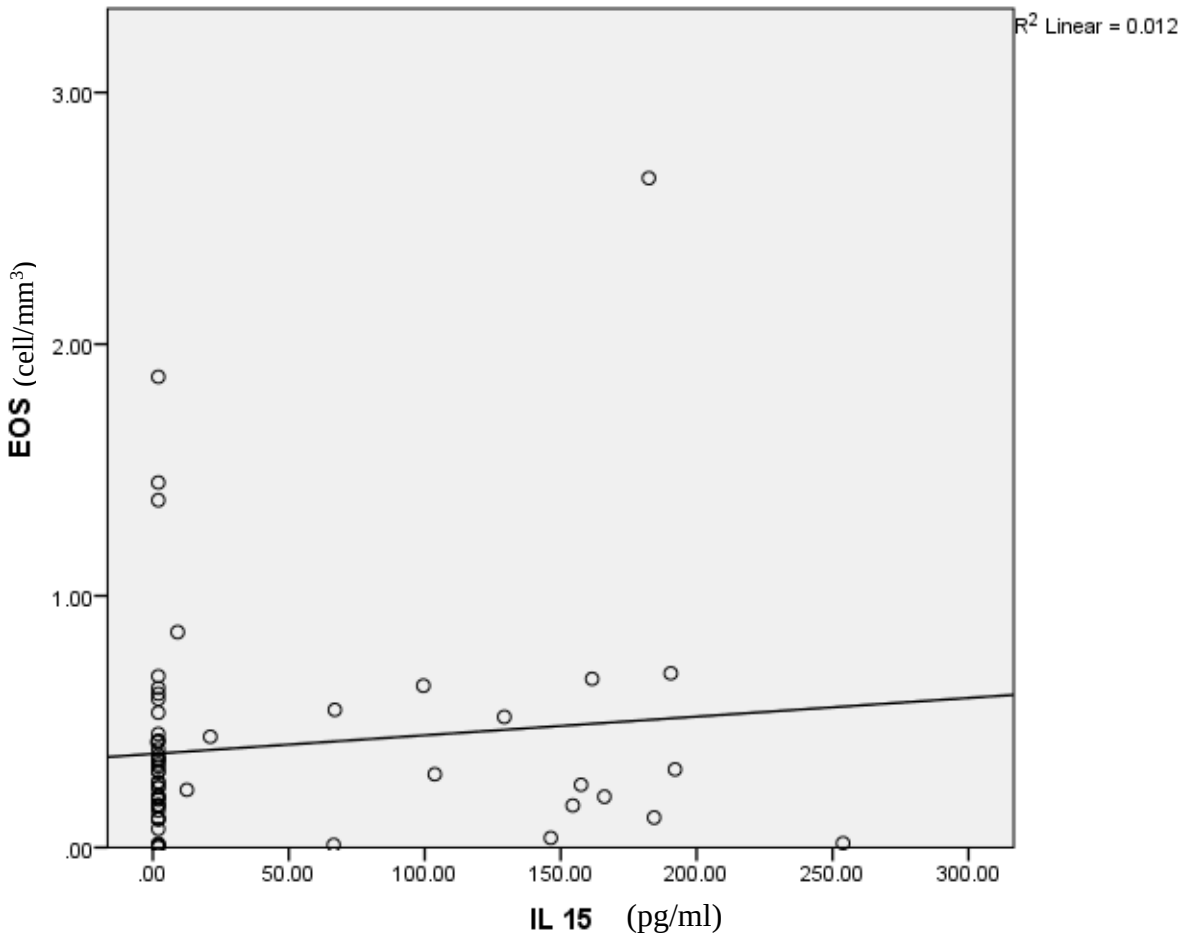

Figure (3-6): Correlation of IL-15 serum level with the eosinophil count pg/ml. Pearson correlation coefficient: r= +0.112 (*p = 0.395*).

3.4.2.2. The Correlation between IL-15 and IgE serum levels in asthmatic patients

Figures (3-7) showed the correlation between the IL-15 and IgE serum levels. No significant negative correlation were found between IL-15 and IgE serum levels, r= -0.136 (*p = 0.301*)**.**

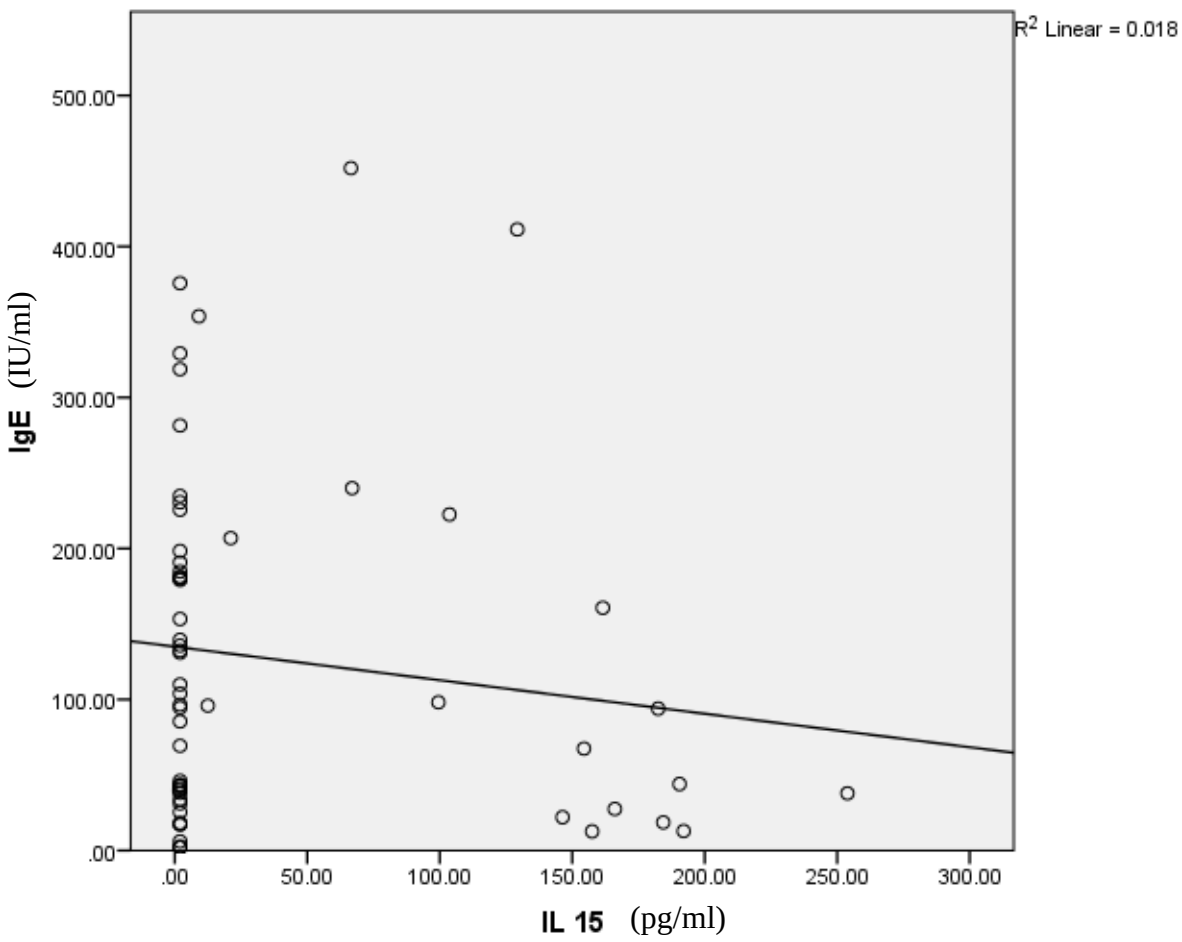

Figure (3-7): Correlation of IL-15 serum level with the serum IgE pg/ml. Pearson correlation coefficient: r= -0.136 (*p = 0.301*).

3.4.3. Correlation of IL-22 serum level and immunological parameters

3.4.3.1. The correlation between IL-22 serum level with Eosinophil Count in asthmatic children

Figure (3-8) showed the correlation between the IL-22 serum level and the eosinophil count in asthmatic children. No significant positive correlation were found between IL-22 and eosinophil count, r= +0.017 *(p-value= 0.900)*.

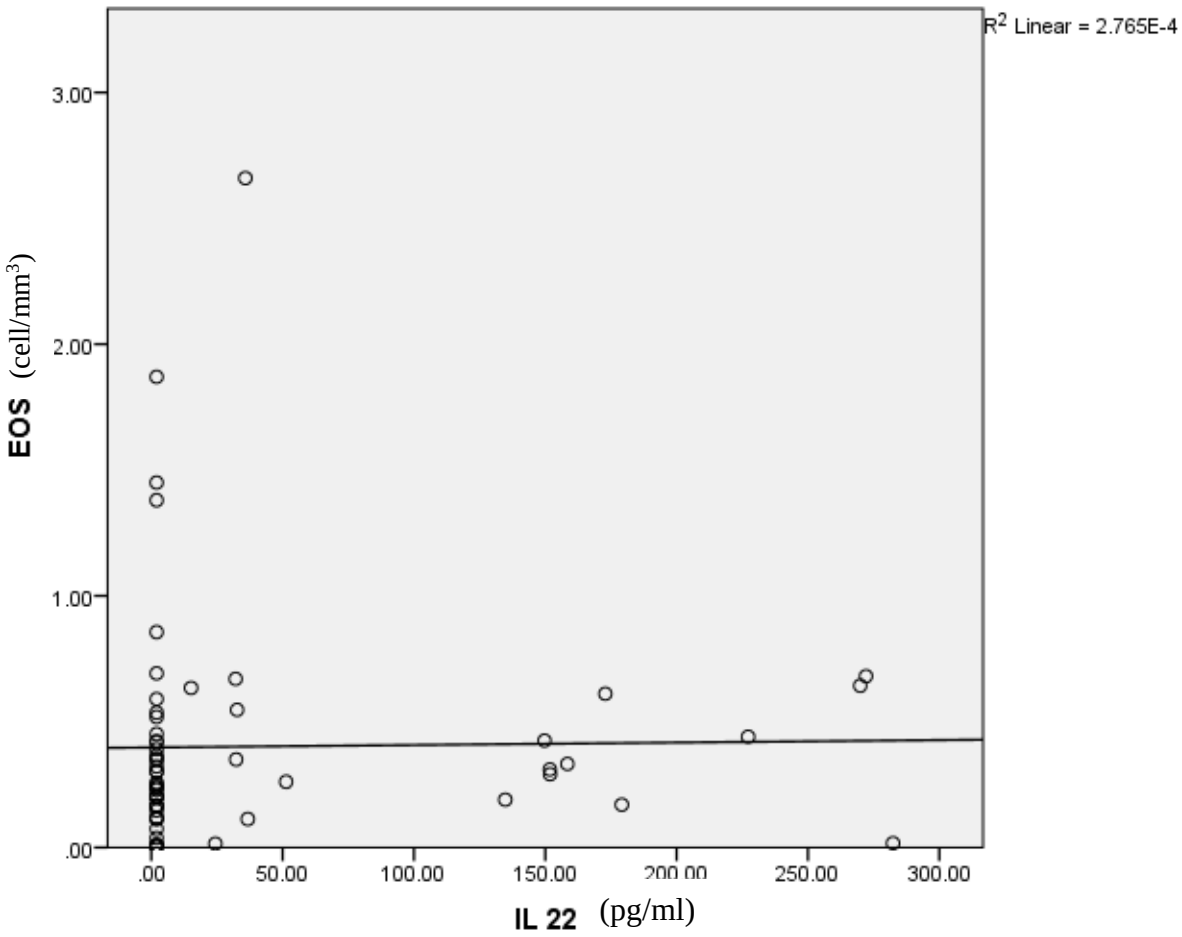

Figure (3-8): Correlation of IL-22 pg/ml serum level with the eosinophil count. Pearson correlation coefficient: r= +0.017 *(p-value= 0.900)*.

3.4.3.2. The correlation between IL-22 and IgE serum level in asthmatic children

Figures (3-9) showed the correlation between the IL-22 and IgE serum levels. No significant negative correlation were found between IL-22 and IgE serum levels, r= -0.040 *(p-value= 0.762)*.

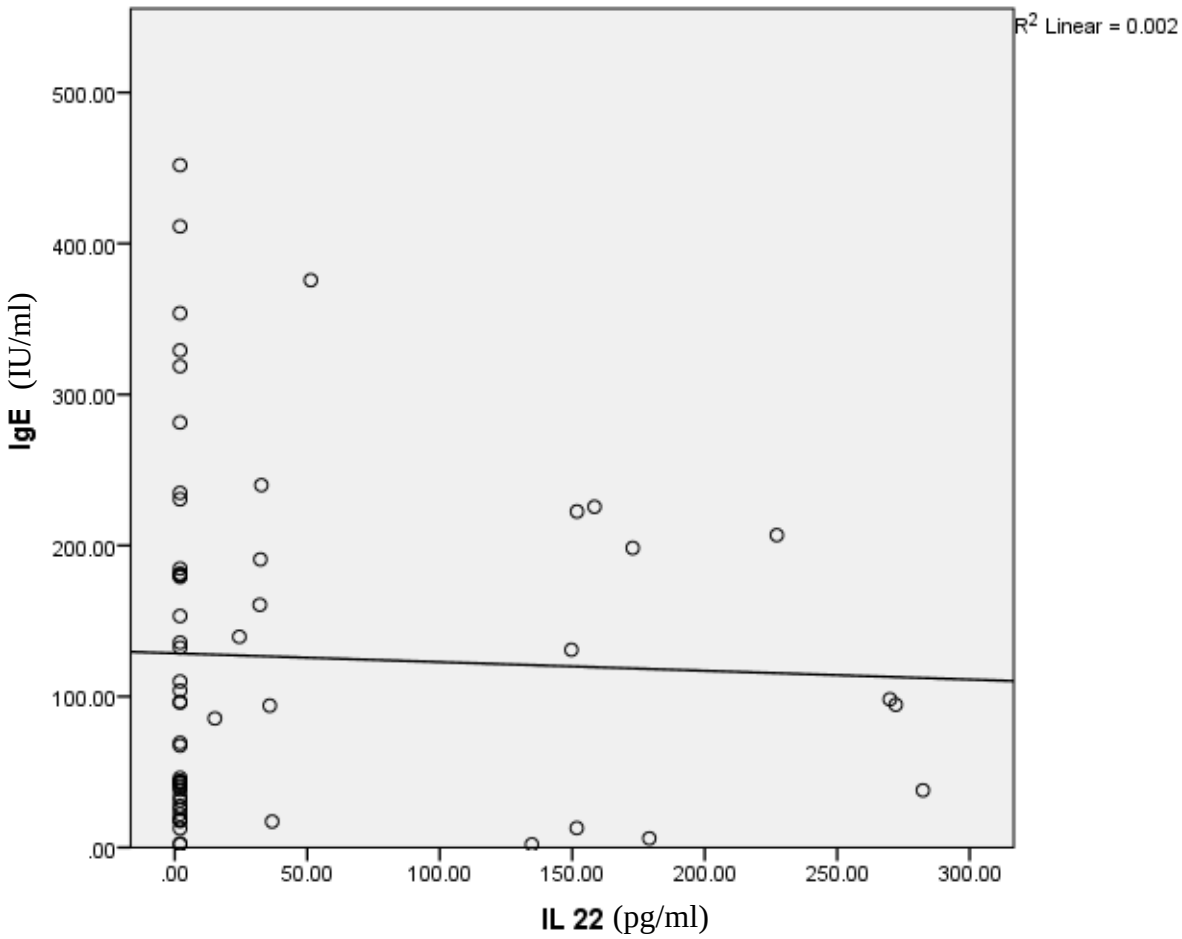

Figure (3-9): Correlation of IL-22 pg/ml serum level with the serum IgE. Pearson correlation coefficient: r= -0.040 *(p-value= 0.762)*.

3.4.3.3. The correlation between IL-22 and IL-15 serum levels in asthmatic children

Figure (3-10) showed the correlation between the IL-22 and IL-15 serum levels. No significant positive correlation were found between IL-22 and IL-15 serum levels, r= +0.215 *(p-value= 0.0.99).*

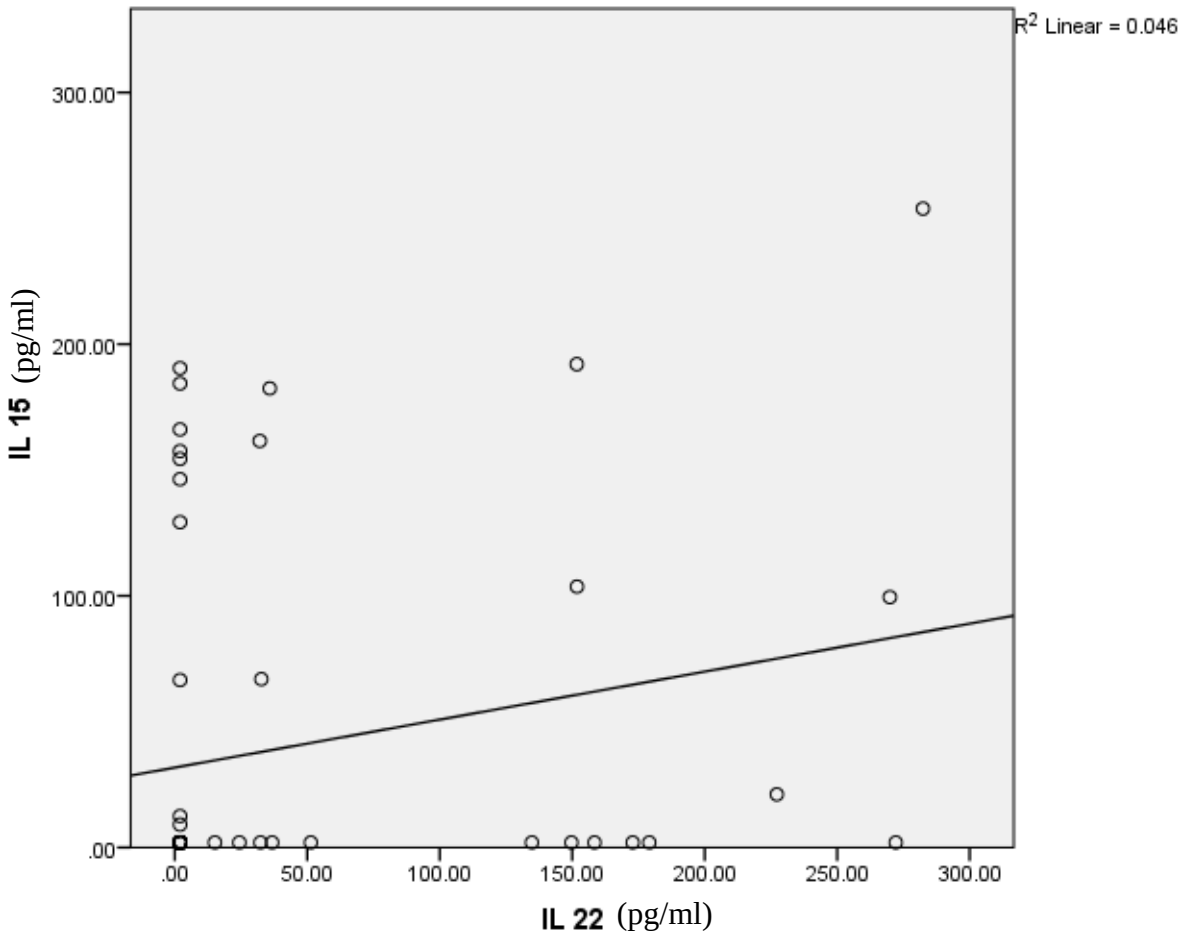

Figure (3-10): Correlation of IL-22 serum level with the serum IL- 15 pg/ml. Pearson correlation coefficient: r= +0.215 *(p-value= 0.0.99).*

3.4.3.4. The correlation between IL-22 and IL-23 serum levels in asthmatic children

Figure (3-11) showed the correlation between IL-22 and IL-23 serum levels. No significant positive correlation were found between IL-22 and IL-23 serum levels, r= +0.081 *(p-value = 0.538).*

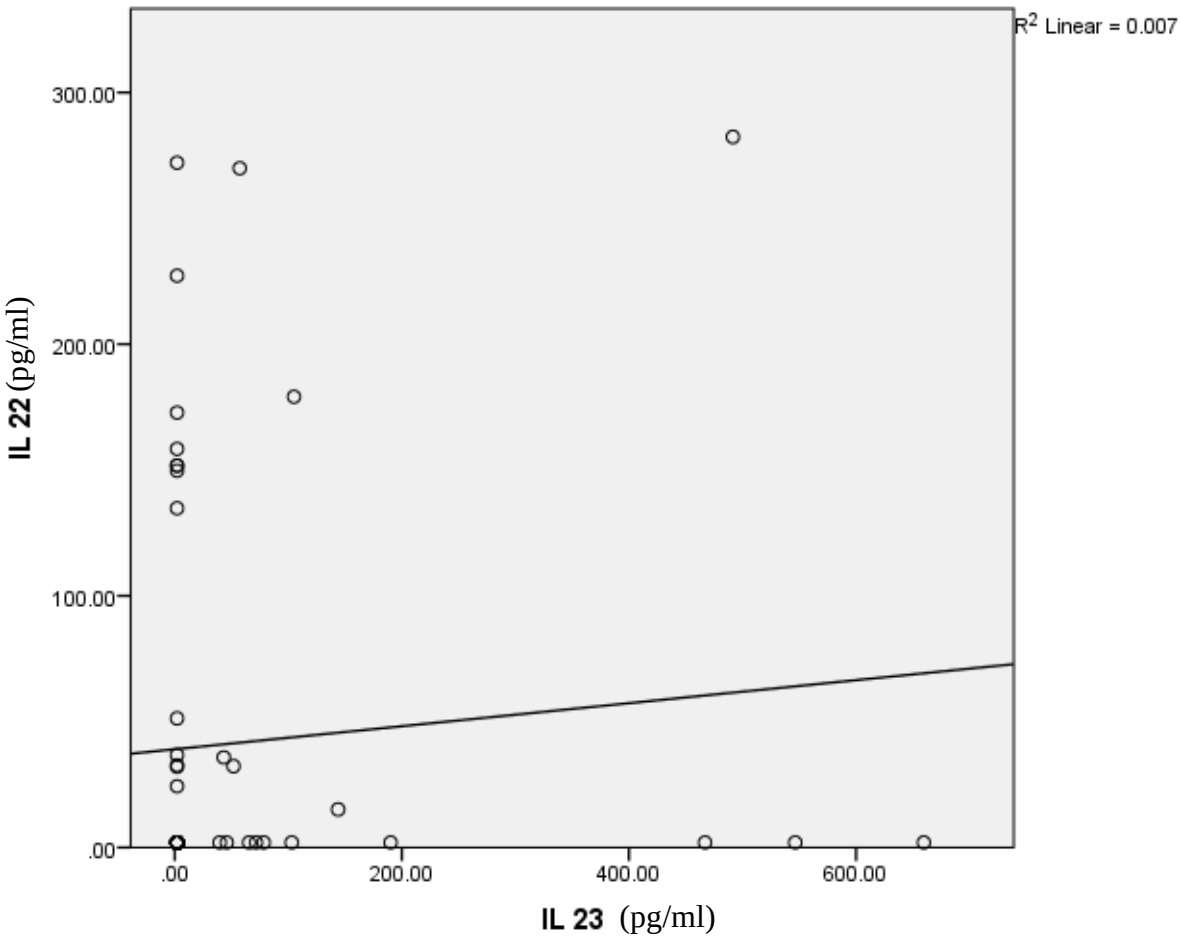

Figure (3-11): Correlation of IL-22 serum level with the serum IL-23 pg/ml. Pearson correlation coefficient: r= +0.081 *(p-value= 0.538).*

3.4.4. Correlation of IL-23 serum level and immunological parameters

3.4.4.1. The Correlation between IL-23 serum level and eosinophil count in asthmatic children

Figure (3-12) showed the correlation between IL-23 serum level and eosinophil count in asthmatic children. No significant positive correlation were found between IL-23 serum level and eosinophil count, r= +0.064 *(p-value= 0.625).*

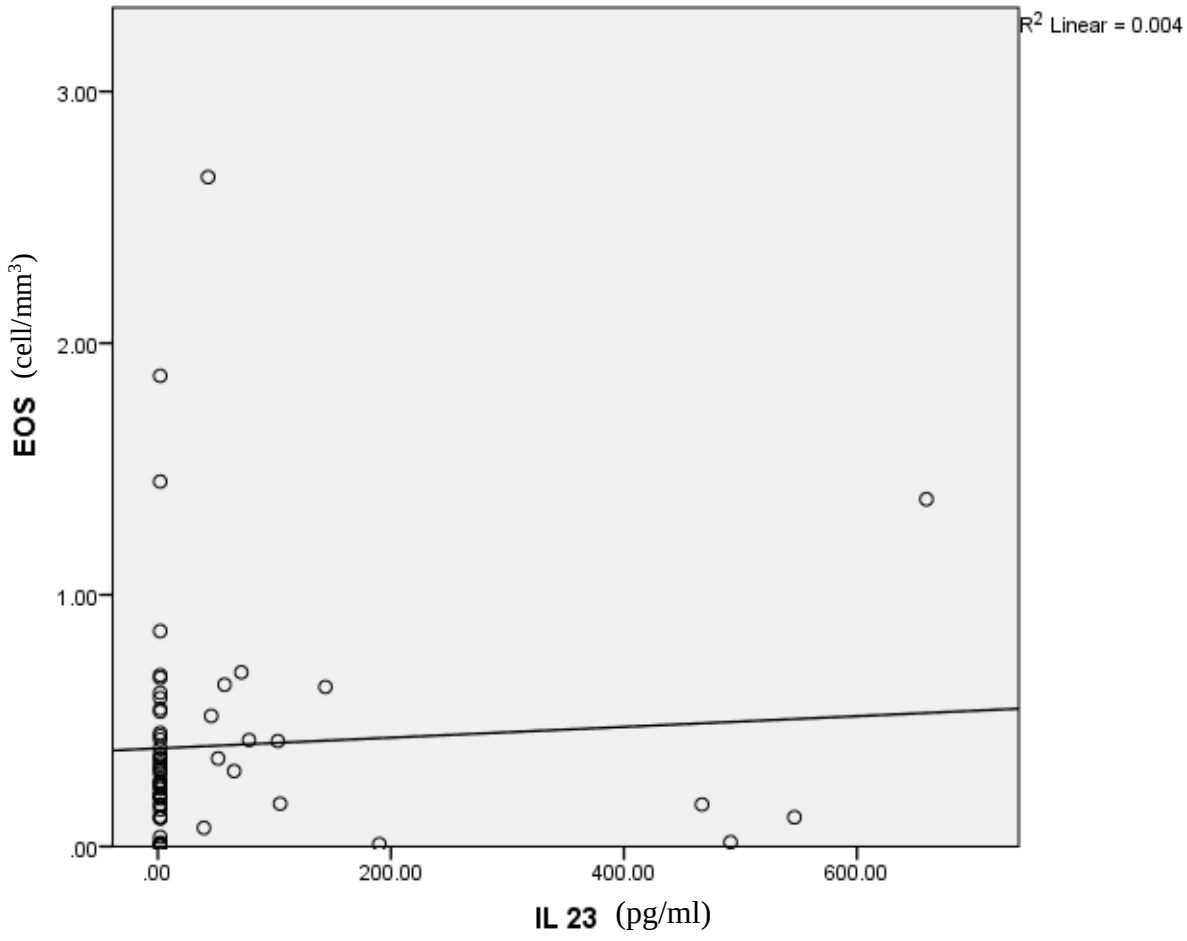

Figure (3-12): Correlation of IL-23 pg/ml serum level with the eosinophil count. Pearson correlation coefficient: r= +0.064 *(p-value= 0.625).*

3.4.4.2. The Correlation between IL-23 and IgE serum levels in asthmatic children

Figure (3-13) showed the correlation between IL-23 and IgE serum levels. No significant negative correlation were found between IL-23 and IgE serum levels, r= -0.170 *(p-value= 0.194).*

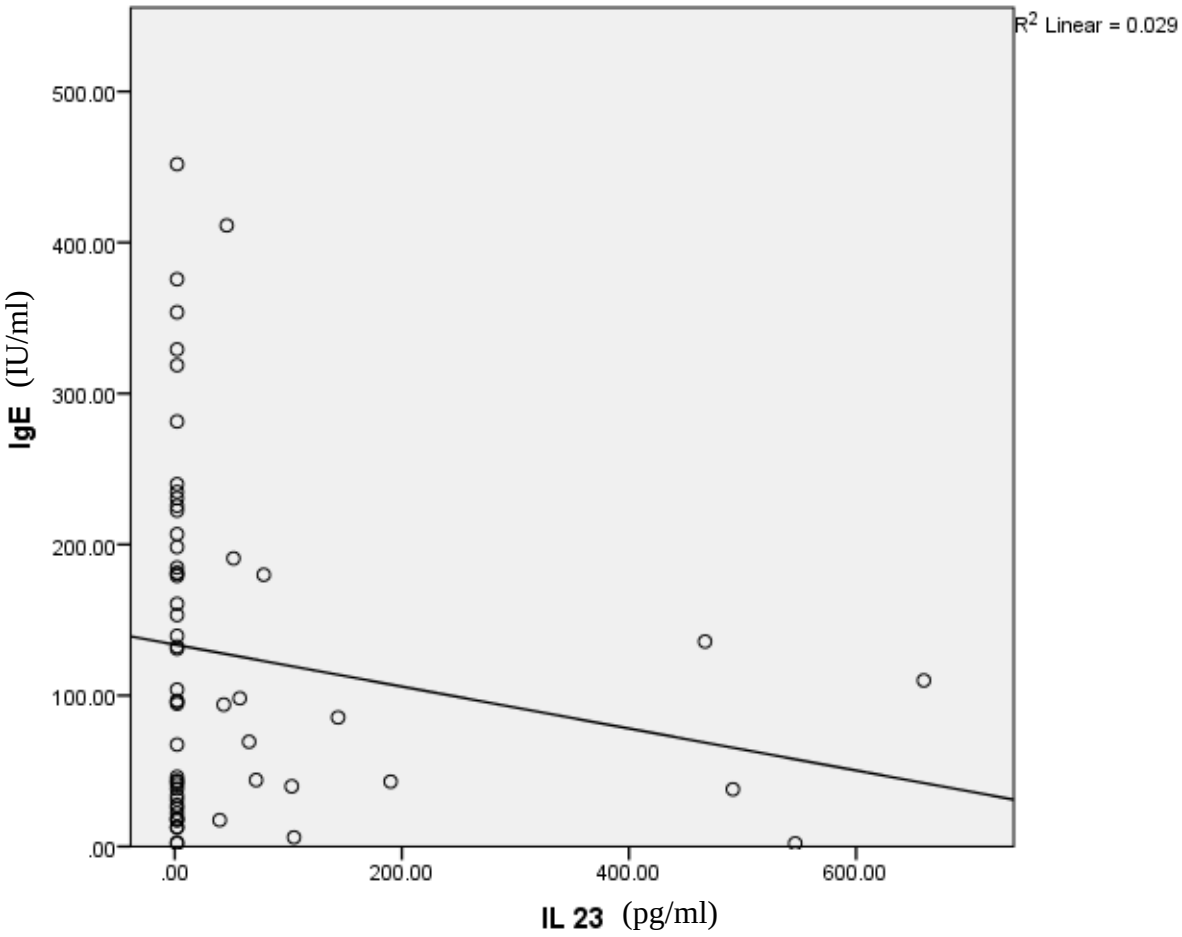

Figure (3-13): Correlation of IL-23 pg/ml serum level with the serum IgE. Pearson correlation coefficient: r= -0.170 *(p-value= 0.194).*

3.4.4.3. The Correlation between IL-23 and IL-15 serum levels in asthmatic children

Figure (3-14) showed the correlation between IL-23 and IL-15 serum levels. No significant positive correlation were found between IL-23 and IL-15 serum levels, r= +0.66 *(p-value= 0.618).*

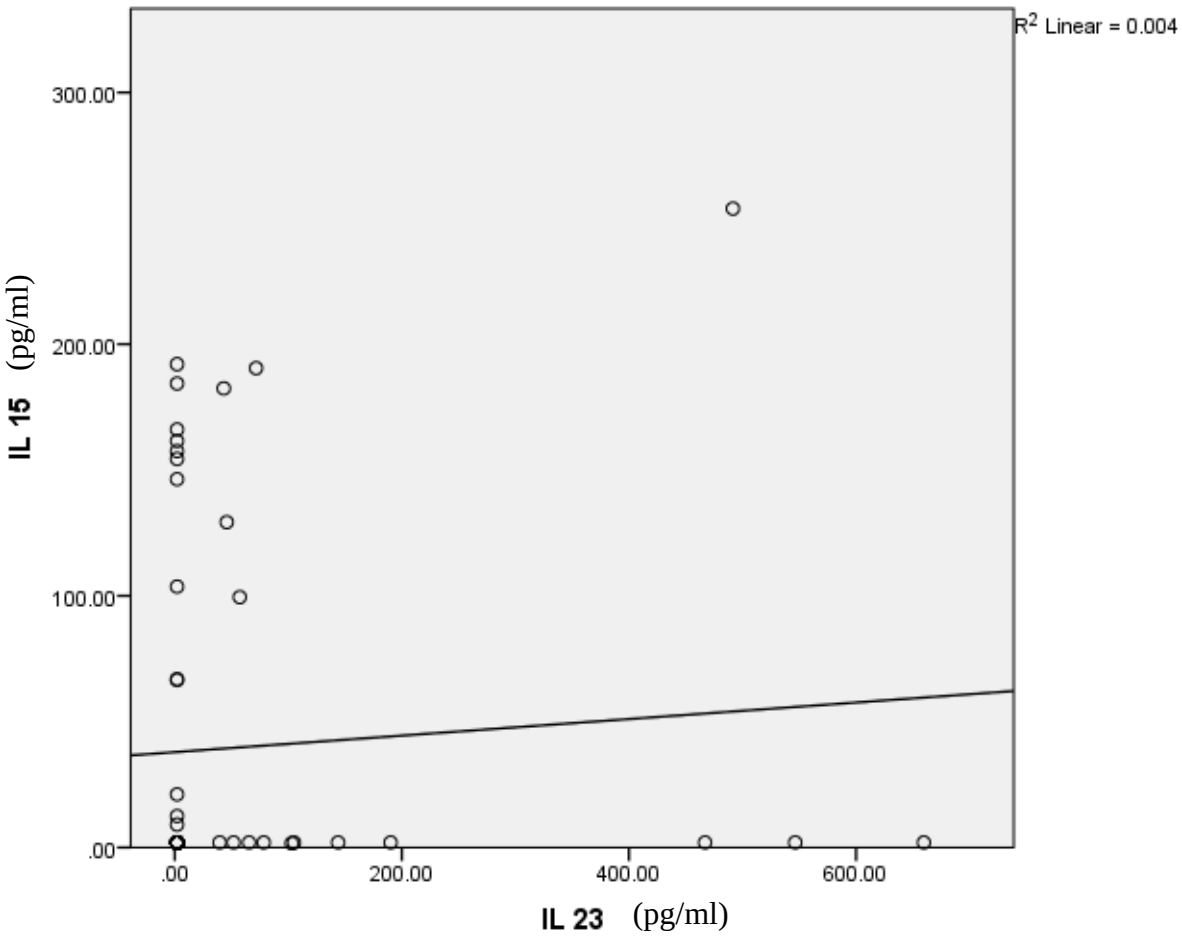

Figure (3-14): Correlation of IL-23 pg/ml serum level with the serum IL-15. Pearson correlation coefficient: r= +0.66 *(p-value= 0.618).*

3.5. Association between asthma severity and immunological parameter in asthmatic children

The current study showed a non-significant difference in the severity of asthma regarding the EOS count, IgE, IL-15, IL-22 and IL-23 levels between the mild and moderate asthmatic children with (*P-value* = 0.344, 0.391, 0.283, 0.247 and 0.441) respectively and mean about (0.30, 154.32, 57.16, 20.28 and 28.99) in the mild asthma compared with mean about (0.43, 117.65, 34.37, 48.01 and 61.77) in the moderate asthma respectively. While, the present study showed a significant difference (*P value*=0.046) in the severity of asthma depending on the duration which was higher in moderate asthma than the mild asthmatic patients with a mean (36.70) and (17.86), respectively, as clarified in table (3-5).

Table 3-5: The relation of the markers and duration with the severity of asthma

Variables	**Severity**				***P-value***
	Mild		**Moderate**		
	Mean	**SE**	**Mean**	**SE**	
EOS	0.30	0.06	0.43	0.07	0.344
IgE	154.32	38.94	117.65	14.92	0.391
IL 15	57.16	18.94	34.37	10.08	0.283
IL 22	20.28	16.07	48.01	12.09	0.247
IL 23	28.99	14.41	61.77	22.78	0.441
Duration	17.86	4.34	36.70	4.90	**0.046***

*** *p-value* is significant*(P<0.05)*, Student's t-test**

3.6. The Relation of the Immunological markers with the presence of aggravating factors in asthmatic patients

This study showed a non-significant association between EOS count, IgE and immunological markers (IL-15, IL-22and IL-23) serum levels with the aggravating factors (*P value*= 0.182, 0.927, 0.981, 0.445 and 0.480) respectively, with a mean about (0.45, 127.06, 39.82, 46.41 and 46.13) respectively regarding the positive result of aggravating factors, compared with mean about (0.28, 124.06, 39.36, 29.21 and 74.33) respectively concerning the negative result of aggravating factors, as demonstrated in table(3-6).

Table 3-6: The relation of the markers with the presence of aggravating factors

Variables	**Aggravating**				***P-value***
	+ve		**-ve**		
	Mean	**SE**	**Mean**	**SE**	
EOS	0.45	0.08	0.28	0.04	0.182
IgE	127.06	17.71	124.06	26.07	0.927
IL 15	39.82	10.58	39.36	17.06	0.981
IL 22	46.41	12.96	29.21	13.67	0.445
IL 23	46.13	19.36	74.33	40.05	0.480

Student's t-test

3.7. The relation between the immunological markers and the asthma phenotypes

There was a significant difference in the EOS count according to each asthma phenotype in the current study; it was the highest in allergic asthma, which was (1.2518), then in the early wheeze, which was (0.4054), and Late-onset asthma which was (0.3237) followed by non-allergic asthma which was (0.3050) than in the persist wheezer which was (0.2699). The lowest was in the adolescent &obese asthmatic patients who were (0.2509) (*P value*= 0.004).

Also, this study showed a non-significant difference in the IgE, IL-15, IL-22, and IL-23 according to each asthma phenotypes with (*P value*=0.954, 0.561, 0.171 and 0.437) respectively, as shown in table (3-7).

Table 3-7: The relation of the markers with the asthma phenotypes

Variables	Asthma phenotype	N	Mean	SE	*P-value*
EOS	Early wheeze	27	0.4054	0.08189	0.004*
	Allergic asthma	4	1.2518	0.52696	
	Non-allergic	4	0.3050	0.09696	
	Adolescent & obesity	8	0.2509	0.06455	
	Late-onset	6	0.3237	0.11002	
	persist wheezer	11	0.2699	0.04847	
IgE	Early wheeze	27	111.1132	16.67824	0.954
	Allergic asthma	4	155.3370	36.10537	
	Non-allergic	4	118.3423	86.82621	
	Adolescent & obesity	8	144.6100	51.68087	

	Late-onset	6	143.6188	68.98339	
	persist wheezer	11	132.6375	36.01495	
IL 15	Early wheeze	27	27.1037	12.10754	0.561
	Allergic asthma	4	63.3378	42.54385	
	Non-allergic	4	81.1328	45.74924	
	Adolescent & obesity	8	40.6656	25.87710	
	Late-onset	6	68.2250	33.76954	
	persist wheezer	11	30.6411	19.21498	
IL 22	Early wheeze	27	71.7393	19.73036	0.171
	Allergic asthma	4	25.7030	7.94006	
	Non-allergic	4	14.3318	12.33175	
	Adolescent & obesity	8	20.4626	18.46262	
	Late-onset	6	2.0000	.00000	
	persist wheezer	11	19.9635	12.13222	
IL 23	Early wheeze	27	95.8063	37.48886	0.437
	Allergic asthma	4	24.7128	13.23038	
	Non-allergic	4	2.0000	0.00000	
	Adolescent & obesity	8	43.6098	24.51394	
	Late-onset	6	24.1775	14.04945	
	persist wheezer	11	5.4108	3.41082	

**** p-value* is significant *(P < 0.05)*, ANOVA test**

3.8.The treatment effect on immunological markers

The current study showed a non-significant differences regarding the treatment effect on EOS count, IgE, IL-15, IL-22 and IL-23 with (*P value*= 0.376, 0.444, 0.407, 0.334 and 0.692) respectively. The EOS count was the highest mean in the patients treated with ICS, which was (0.62) followed by the patients with mixed treatment, which was (0.54) then the patients treated with montelukast, which was (0.35) and the lowest mean in patient whose haven't receive any treatment (0.34). The IgE level was the highest mean in the patients controlling their asthma with Montelukast, which was (149.44), followed by the patients with ICS treatment which was (104.66) then the patients treated with mixed treatment, which was (100.31). The lowest mean in patients who haven't received any treatment was (99.56).

Regarding the IL-15 level was the highest mean in the patients controlling their asthma with mixed treatment, which was (81.62) followed by the patients who hadn't had any treatment, which was (57.91) then the patients treated with ICS treatment which was (39.41). The lowest mean in patients controlling their asthma with montelukast treatment was (27.09). The IL-22 level was the highest in the patients controlling their asthma with Montelukast, which was (55.74) followed by the patients with ICS treatment which was (49.18), then the patients treated with mixed therapy which was (17.05). The lowest mean in patients who haven't received any treatment was (14.03). Concerning the IL-23 level was the highest mean in the patients controlling their asthma with Montelukast, which was (69.40) followed by the patients with ICS treatment which was (65.52) then the patients treated with mixed treatment which was (52.49) and is the lowest in patient whose haven't receive any treatment which was (19.74), as found in table (3-8).

Table 3-8: The treatment effect on markers

Variables	Treatment								*P-value*
	No treatment		Montelukast		ICS		Mixed		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
EOS	0.34	0.11	0.35	0.05	0.62	0.26	0.54	0.13	0.376
IgE	99.56	29.05	149.44	21.51	104.66	23.42	100.31	60.42	0.444
IL-15	57.91	19.18	27.09	10.79	39.41	22.60	81.62	79.94	0.407
IL-22	14.03	8.16	55.74	16.87	49.18	24.39	17.05	15.05	0.334
IL-23	19.74	11.63	69.40	29.19	65.52	53.76	52.49	50.49	0.692

3.9.Receiver operating characteristic curves (ROC)

ROC curve analysis showed cutoff value of (0.11) for Eos with AUC (0.156) , (40) for IgE with AUC (0.773) , (1.84) for IL-15 with AUC (0.722) , (1) for IL-22 with AUC (0.767) and also (1) for IL-23 with AUC (0.943) . According to the AUC, the IL-23 was a predictive marker for diagnosing pediatric asthma as shown in table (3-9) . The sensitivity and specificity of immunomarkers were clarified in table (3-10).

Table 3-9: Diagnostic utility of area under the curve in figure 3-15

Area Under the Curve (AUC)

Test Result Variables	Area	Std. Error[a]	*P value*[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
EOS	0.156	0.038	0.0005	0.081	0.230
IgE	0.773	0.042	0.0005	0.690	0.855
IL 15	0.722	0.053	0.0005	0.618	0.825
IL 22	0.767	0.051	0.0005	0.667	0.867
IL 23	0.943	0.028	0.0005	0.889	0.997

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

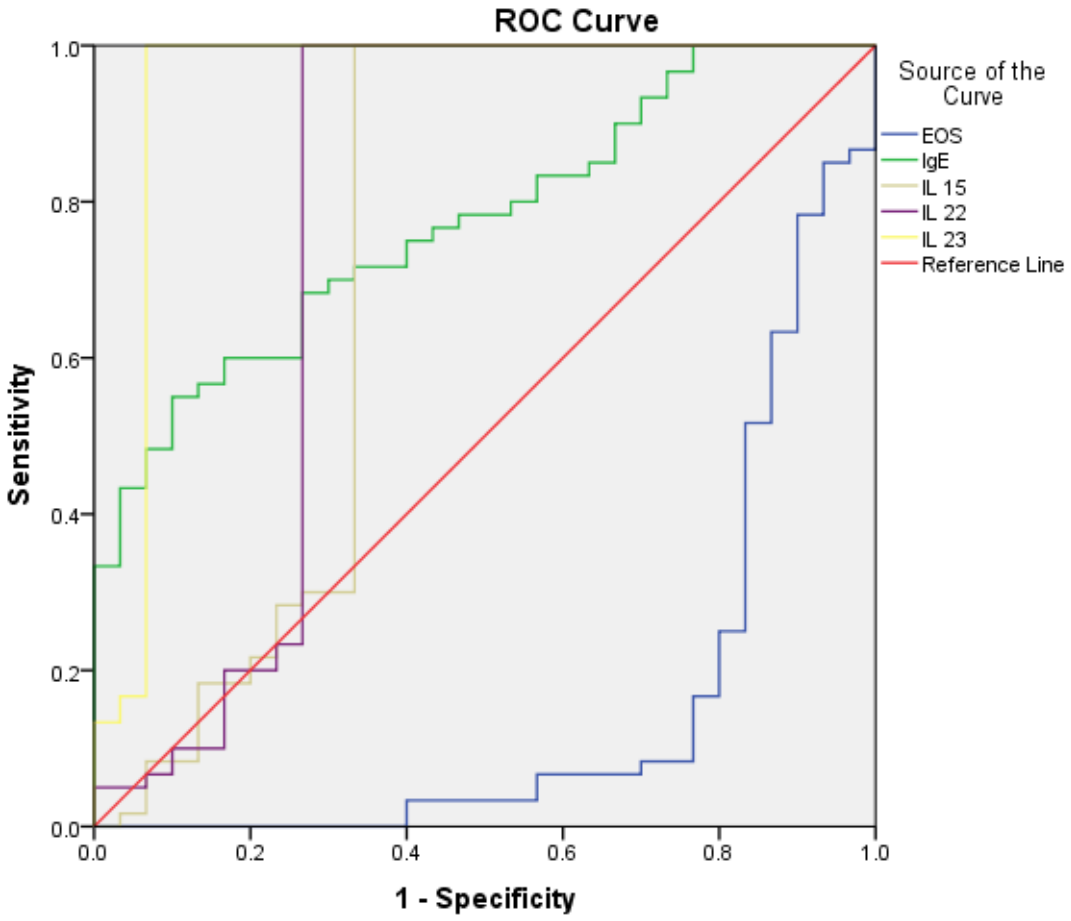

Figure (3-15): Receiver operating characteristic curves of IL-15, IL-22, IL-23, IgE and eosinophil count for diagnosis of asthma

Table 3-10: Cut-off values, sensitivity and specificity in differentiating patients from the control groups

Variables	Cut-off value	Sensitivity	Specificity
EOS	0.11	85.0	6.7
IgE	40.0	70.0	70.0
IL-15	1.84	98.3	62.7
IL-22	1.00	100.0	75.3
IL-23	1.00	100.0	93.3

Chapter four
Discussion

4. Discussion

4.1. Demographic data among patients and control:

The current study showed that there was no significant difference*(p=0.413)* in age between the patients and control groups; this result was agreed with a study achieved by Zheng *et al.,* (2020) indicated no significant differences in age among asthmatic children and control.

The present study showed that males suffered from asthma more than females. The majority of the patient group were males 35 (58.3%) compared with 25 (41.7%) females. This result was associated with a study conducted by Hameed *et al.,* (2019) who revealed that the presence of pediatric asthma was higher in males which were (69.4 %) compared with females (30.6%). Also, a study conducted by (Wright *et al.*, 2006) showed that boys were significantly more likely than girls to have asthma (53.8% vs. 43.3%). Also Zein and Erzurum, (2015) revealed that the onset of asthma is more in boys than girls in childhood. The mechanism of the changing gender ratio appears to be the late incidence of asthma among girls because the latter constitute a considerable part of adult asthma cases (Nicolai *et al.*, 2003).

Regarding the history of eczema, the present study found 2(3.3%) of asthmatic patients had a positive result compared to 1(1.7%) of control. In the same line Von Kobyletzki *et al.*, (2012) revealed that children with eczema at baseline had more than a 2-fold increase in the odds of developing asthma. However, in this study the association was not highly significant the differences of these results may be due to the ethnicity in different populations.

Regarding the family history of eczema, the present study found that 7(11.7%) of asthmatic patients had a positive family history of eczema result compared with 0 (0%) of controls. This study detected the family history of eczema was effective

in asthmatic patients, the association was significant between family history of eczema and pediatric asthma (P=0.013). In the same line Spergel, (2010) showed that eczema was demonstrated to be a major risk factor for the development of asthma and hay fever. This might be due to genetic predisposition. Weidinger *et al.*, (2008) demonstrated that Filaggrin (flg) must be considered to be a major gene in the development of eczema, atopic sensitization, and allergic rhinitis, with a significant effect in asthma that is restricted to patients with a history of eczema.

About family history of rhinitis, the current study found that 30 (50%) of asthmatic patients had a positive result compared with 8 (13.3%) of controls. The result showed a high association between family history of rhinitis and asthma. Bergeron and Hamid, (2005) showed that Allergic rhinitis is an important risk factor for developing asthma and is also an important cause of nonoptimal control of asthma. Some other studies compatible with result of this study, showed the family history of rhinitis risk factor for asthma (Dold *et al.*, 1992; Gelardi *et al.*, 2014).

Regarding the Allergic conjunctivitis, the present study found that17 (28.3%) of asthmatic patients had a positive result compared with 8(13.3%) of controls. Agreed with Michailopoulos *et al.*, (2017) who showed that Allergic conjunctivitis was found to be very common (40%, 497 out of 1242 patients) among those with symptoms of allergies. In the same line Gradman and Wolthers, (2006) whose found (33%) of asthmatic patients have allergic conjunctivitis and suggested adding allergic conjunctivitis as an important co-morbidity in future guidelines on asthma.

There was a highly significant association (*P*=0.001) between exposure to cigarette smoke and pediatric asthma appeared 30 (50%) of patients had positive exposure to cigarette smoke compared to 12(20%) of control. This result was in line He *et al.*, (2020), found out that there were significantly positive associations

between second hand smoking SHS exposure and doctor-diagnosed asthma and asthma-like syndrome. In the same line Jing, Wang and Liu, (2019), found out that Passive smoking was closely associated with the severity of childhood asthma by affecting the balance of Treg/Th17 cell.

4.2. Mean differences serum levels of immunological markers

In the present study there was a significant association *(P= 0.005)* between eosinophils count in control compared with asthmatic patient as shown in table 3-2. Other studies indicated a non-significant eosinophil count in asthmatic patients such as a study achieved by Ullmann *et al.*, (2013) who showed that (86%) of asthmatic patient had normal eosinophils count. Also, Amin *et al.*, (2000) showed the low number of eosinophils in non-atopic asthma. Contrary to current study, Foster *et al.*, (2008) revealed that recruitment of eosinophils has long been recognized as a hallmark of the inflammatory response in asthma. The differences might be due to the small samples size and different procedures used.

In this study there was a significant association *(P=0.005)* between serum IgE level in asthmatic patients compared with control. This result related with previous studies such as a study achieved by Strømgaard *et al.*, (2011), who found a strong positive relationship between total serum IgE level and asthma in children**.** Also, a study achieved by Maneechotesuwan, Sujaritwongsanon and Suthamsmai, (2010) Whose revealed that serum total IgE concentrations were significantly higher in patients with uncontrolled asthma. In the same line Abood, Ghazal and Al-Musawi, (2013) mentioned that (48.7%) of asthmatic patients showed positive IgE screening.

Regarding the IL-15 serum level, this study found that there was no significant differences between asthmatic patients and control *(P value = 0.968*). This result agreed with a study achieved by Stoner *et al.*, (2019) who showed a similar IL-15

responses in exacerbating asthmatics and control. Contrary to this result, a study conducted by Bierbaum *et al.*, (2006) who confirmed the association of IL-15 with asthma by conducting a genotyping of all IL-15 polymorphisms within the promotor and coding region of IL-15 which were identified by single strand conformation polymorphism (SSCP) analyses and sequencing in the initial study.

About the IL-22 serum level, this study found that there is no significant differences between asthmatic patients and control *(P value = 0.721).* Several studies showed a contrary result such as a study achieved by Besnard *et al.*, (2011) who showed that IL-22 play a crucial role in antigen sensitization in a murine asthma model. And a study achieved by Zhu *et al.*, (2011) who demonstrated that IL-22 level was increased in the sera of asthma patients and were positively correlated with disease severity. The differences of these results may be due to the ethnicity in different populations.

In addition, the present study showed a significant association (*P=0.008)* between the IL-23 serum level and asthmatic patients compared with control. This result was agreed with several studies, such as a study established by Ciprandi *et al.*, (2012), who revealed that IL-23 levels were higher in asthmatic patients than in healthy children ($p < 0.001$) and who also suggested that the serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children.

4.3. Association of severity with aggravating factors, treatment and phenotypes in asthmatic patients

The patients in the present study were classified into two groups, mild and moderate according to the asthma severity. Number of patients having moderate asthma was higher than number of patients with mild asthma, as in table(3-3).This results agreed with a previous study achieved by Hinks *et al.*, (2015) who

demonstrated that the moderate asthma more prevalent than mild and severe asthma and a study conducted by Dolan *et al.*, (2004)who showed that percentage of moderate asthma was (48%) more than mild but equal with severe asthma, while other studies reported by Jabbar, (2011); Fleming *et al.*, (2015) and Abood and Mohanad, (2018) inconsistent with current study. The differences of these results may be due to these studies were designed with different methodologies and the selection of their study population was not randomized.

About aggravating by pets, there was no significant differences between mild and moderate asthmatic patients regarding aggravating by cats *(p*=0.427), this study was inconsistent with a result of Simoneti *et al.*, (2018) who demonstrated that exposure to cats were associated with increased risk of asthma. Also, this study showed no significant differences *(P=0.585), (P*=0.727*)* between mild and moderate asthmatic patients regarding the aggravating by dogs and birds respectively. This study was incompatible with a study conducted by Weber-Chrysochoou *et al.*, (2014), whose confirmed that birds may exacerbate asthma severity. This variety may be attributable to the difference in sample group or differences in study geographic location and also socio-cultural variations of study subjects.

This study found a significant association between aggravation by upper respiratory inflammation and mild asthma compared with moderate asthma (*P=0.031)*. This study related with Grissell *et al.*, (2005), who found that respiratory infection can provoke asthma. In the same line, a study was achieved by Corne *et al.*, (2002), who confirmed that upper respiratory inflammation causes an extended duration of illness and increased severity of lower respiratory symptoms in individuals with asthma.

In this study there was no significant differences in the severity of asthma regarding the aggravation by dust between mild and moderate asthma (*P= 0.535*). On the contrary to this result a study achieved by Cadelis, Tourres and Molinie, (2014) whose revealed to that dust represent a high risk to asthma and increase severity of asthmatic patients. This discrepancy might be attributed to the differences in sample size between this study and theirs.

In this study, there were no significant differences in the severity of asthma regarding aggravating by physical activities *(P= 0.379*). This study was inconsistent with a study reported by Paggiaro and Bacci, (2011) whose revealed that exercise-induced bronchospasm is frequent in children and in young patients with mild asthma.

Regarding the controlling therapy, this study revealed to a significant association (*P = 0.049)* between the no treatment administrating and the mild asthma compared with moderate asthma, and an association between the patients who were controlling their asthma with montelukast and the moderate asthma compared with the mild asthma. The result of present study was compatible with a study accomplished by Paggiaro and Bacci, (2011) whose reported that montelukast improved symptoms, rescue medication use and pulmonary function, and reduced the rate of exacerbation and the level of blood eosinophils, in mild-to-moderate asthmatics not treated with ICS.

Regarding asthma phenotype, there were no significant differences (*P= 0.991*) between asthma phenotype and disease severity. Fitzpatrick and Moore, (2017) found that despite global differences between severe and non-severe asthma in Severe Asthma Research Program (SARP), significant heterogeneity was present in both groups, prompting further exploration of phenotypes irrespective of asthma severity definitions.

4.4. Correlation between the studied markers

4.4.1. Correlation of Eosinophils, IgE and immunological markers with the duration in asthmatic patients

This study showed no significant correlation were found between EOS count and duration of disease (*p = 0.646*) as in figure 3-1. The decrease in EOS count might be due to ICS administration. A suppressed EOS was associated with the treatment effect of ICS Mathioudakis *et al.*, (2020) or it might be because of asthma aggravation. This result disagreed with a study achieved by Hancox, Pavord and Sears, (2018) whose assured that increased Blood eosinophils are associated with airflow obstruction and enhanced decline in lung function.

This result showed no significant association regarding the IgE serum level and duration of asthma (*P-value = 0.898*) as in figure 3-2. This result in the same line with a study conducted by Mediaty and Neuber, (2005) whose found that total serum IgE was significantly decreased as a function of age in patients with asthma. Also similar to a study conducted by Ahmad Al Obaidi *et al.*, (2008), who demonstrate that specific immunotherapy reduced total IgE serum level in 36% of patients with asthma.

About IL-15, this result showed no significant association between the IL-15 serum level and duration of asthma (*p = 0.675*) as in figure 3-3. This might be because of treatment admission, a study conducted by Komai-Koma *et al.*, (2001) confirmed that IL-15 production appears to be reciprocally regulated by steroid therapy in asthma patients.

Concerning IL-22, this study result showed no significant association between duration of asthma and IL-22 serum level (*p=0.772*) as in figure 3- 4. A study by

Zhao *et al.*, (2010) found that the percentages of Th17 cells and the plasma concentrations of IL-17 and IL-22 tended to increase with the severity of the disease.

The present study also showed a significant negative correlation between the duration of asthma and the IL-23 serum level (*p=0.030*) as in figure 3-5, this might be due to a long-term complication of uncontrolled asthma is airway remodeling and bronchial function impairment (Bhargava *et al.*, 2021). A study conducted by Ciprandi, Cuppari and Salpietro, (2012) who showed that serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children.

4.4.2. Correlation of IL-15 serum level and immunological parameters

This study showed no significant association between IL-15 level and EOS count (*p = 0.395*) with a non-significant positive correlation (r=0.112), this might be as described by a study conducted by Huilan *et al.*, (2010); Zhu *et al.*, (2011), who demonstrated that IL-15 activated STAT5 and CD4+ T cells to produce cytokines that act on eosinophils. Also, Hoontrakoon *et al.*, (2002) found that IL-15 plays an important role in allergic diseases by inhibiting eosinophil apoptosis and that its anti-apoptotic effects appear to be mediated through autocrine production of GM-CSF and ultimately by NF-κB activation.

In addition, this study showed that there was no significant association between IL-15 level and IgE level (*p = 0.301*) with a non-significant negative correlation (r=-0.136). This result was agreed with a study achieved by Ong *et al.*, (2002) who demonstrated that IL-15 suppressed IgE synthesis. Therefore, a decrease in IL-15 may contribute to the elevation of IgE level in atopic disease. Also, this study was compatible with a study confirmed by Huilan *et al.*, (2010) who demonstrate that the decrease of the IL-15 serum level induces the increasing expression of IgE produced by B cells.

About IL-22, this study appeared that there was no significant association between IL-15 and IL-22 serum levels (*p = 0.099*). with a non-significant positive correlation (r= +0.215) , the relation of IL-15 and IL-22 serum level haven't been demonstrated before.

Moreover, this study found that there was no significant association between IL-15 and IL-23 serum levels (*p= 0.618*) with a non-significant positive correlation (r= 0.066) , the correlation of IL-15 and IL-23 serum levels haven't been demonstrated before.

4.4.3.Correlation of IL-22 serum level and immunological parameters

This study showed that there was no significant association between IL-22 serum level and EOS count(*p= 0.900*) with a non-significant positive correlation (r= 0.017), this might be due to a study of Nakagome *et al.*, (2011), who demonstrated a novel role for IL-22 as a broad immune-suppressive cytokine suppressing Ag-induced CD4+ T cell priming. On the contrary, a study conducted by Tamasauskiene *et al.*, (2021) showed that IL-22 level in nasal lavage negatively correlated with eosinophil count in a nasal smear in patients with allergic asthma (r=−0.83, *p<0.05*).

Also, this study showed no significant association between IL-22 and IgE serum levels (*p = 0.762*) with a non-significant negative correlation (r=-0.040). on the contrary to present result, a study conducted by Farfariello *et al.*, (2011) who performed a statistical analysis revealed that total IgE serum level correlate with IL-22 mRNA level in peripheral blood mononuclear cells (PBMCs), and a significant correlation between total IgE serum level and IL-22 mRNA was found. The differences of these results may be due to the ethnicity in different populations. In addition, this study showed a non-significant association of IL-22 with IL-15 and

IL-23 serum levels with a non-significant positive correlation (r= +0.215, *p-value= 0.0.99)* and (r= +0.081, *p-value = 0.538)* respectively.

4.4.4. Correlation of IL-23 serum level and immunological parameters

Regarding the IL-23 serum level, this study showed that there was no significant association between IL-23 level and EOS count *(p-value= 0.625)* with a non-significant positive correlation (r=+ 0.064). A study achieved by Guerra *et al.*, (2017) confirmed that IL-23 heterodimer was indeed produced in acutely infected lungs, and that their levels as measured by ELISA were significantly diminished in the absence of eosinophils . Also confirmed that eosinophils are the predominant local source of IL-23 in this acute aspergillosis model.

Also, this study reported that there was no significant association between IL-23 and IgE serum levels *(p-value= 0.194)* with a non-significant negative correlation (r=- 0.170). IL-23 signaling may regulate allergic asthma through modulation of Th2 cell differentiation Peng *et al.*, (2010) . The Th2 cell differentiation plays a triggering role in the activation and/or recruitment of IgE antibody Maggi, (1998). In addition, this study showed that there is no significant association of IL-23 level with IL-15 serum level with a non-significant positive correlation (r= +0.66 , *p-value= 0.618)*.

4.5. Correlation between asthma severity and immunological parameters in asthmatic children

This study found no significant differences regarding the EOS count between mild and moderate asthma patients (*p = 0.344*) as in table (3-5). On the contrary, (Johansson, 2017) and (Nakagome and Nagata, 2018)found that Eosinophils were likely to contribute to the development of asthma and it was an important factor for predicting asthma exacerbation. Also, a study performed by Shikotra *et al.*, (2012)

and Ko *et al.*, (2021) reported a highly significant association between the severity of asthma and eosinophil count.

Also, this study showed no significant differences regarding the IgE serum level between mild and moderate asthma patients (*p = 0.391*). Increases in IgE serum level could be viral (which is the commonest cause of exacerbation of symptoms in asthma) or allergen-specific or could simply represent a generalized upregulation of IgE production (Corne and Holgate, 1997) . Naqvi *et al.*, (2007) found that higher IgE level is associated with lower baseline lung function and more severe asthma. Also, Kovac *et al.*, (2007) demonstrated that asthmatic children with higher asthma severity have a higher serum concentration of total IgE.

In addition, this study showed no significant differences regarding the IL-15 serum level between mild and moderate asthma patients (*p = 0.283*). Laza-Stanca *et al.*, (2011) found that the deficient of IL-15 production in asthma may be important in the pathogenesis of asthma exacerbations.

Regarding IL-22 serum level, this study showed no significant differences of IL-22 serum level between mild and moderate asthma patients (*p = 0.247*). It has been reported that IL-22 level was increased in the sera of asthma patients and were positively correlated with disease severity (Zhao *et al.*, 2010a; Besnard *et al.*, 2011 and Zhu *et al.*, 2011). A study performed by Farfariello *et al.*, (2011) who demonstrated a positive correlation between the severity of chronic asthma and IL-22 mRNA level, with a significant increase of IL-22 mRNA expression in patients with severe asthma.

Concerning IL-23 serum level, this study reported no significant differences of IL-23 serum level between mild and moderate asthma patients (*p = 0.441*). A study conducted by Ciprandi *et al.*, (2012) on seventy-eight children with asthma

suggested that IL-23 serum level could be a suitable marker of bronchial function impairment in allergic asthmatic children.

Moreover, this study showed a significant association between the duration and moderate severity of asthma ($p = 0.046$). Moore *et al.*, (2010) found that childhood asthma severity is associated with duration of asthma symptoms, medication use, lung function, low socioeconomic status, racial/ethnic minorities, and a neutrophilic phenotype.

4.6. The relation of the Immunological markers with the presence of aggravating factors in asthmatic patients

This result showed no significant association between the EOS, IgE and immunological markers with the presence of aggravating factors as in table (3-6) . Some studies reported the number of Th22 cells and level of serum IL-22 to be significantly higher in patients with dust when compared to healthy individuals. The concentration of Th22 in peripheral blood from patients with allergic rhinitis correlated with nasal congestion and rhinorrhea, while the level of IL-22 was significantly associated with the level of IgE specific to the allergens of house dust mites in patients with allergic rhinitis. (Tamasauskiene and Sitkauskiene, 2018). One of the present study limitations is lake of patients serum levels according to allergy into allergic and non-allergic , which could explain the differences in results from other studies which are specific for allergic asthma and could be associated with increased levels of Eosinophils ,IgE and Cytokines levels.

4.7. The relation of the markers with the asthma phenotypes

Current study found a significant association between the EOS count and allergic asthma ($p=0.004$) as in table (3-7), this agreed with a study conducted by Humbert *et al.*, (1999) who demonstrated that Eosinophilia does not increase in all

cases of asthma; it is more common in allergic asthma. Another study by Syabbalo, (2020a) who found that severe refractory asthma encompasses several cellular and molecular phenotypes of asthma, including eosinophilic, neutrophilic, paucigranulocytic, and mixed cellularity asthma.

Also, this study showed no significant difference regarding IgE and asthma phenotypes (*p=0.954*). Lynch *et al.*, (2014) revealed that the activation of allergen-specific Th2 cells is associated with an amplification of allergen-specific IgE production in a vicious cycle of the pathogenic mechanisms of allergic asthma. In addition, this result showed no significant differences regarding IL-15 and IL-22 (*p=0.561*), (*p=0.171*) respectively with asthma phenotypes.

Moreover, this result revealed that there was no significant difference regarding IL-23 and asthma phenotypes (*p=0.437*). Syabbalo, (2020) had found that serum IL-23 level was increased in allergic asthmatic children not on corticosteroids treatment, compared with non-allergic children, and IL-23 levels were strongly and inversely correlated with lung function (FEV1), and airflow limitation in small airways (FEF25-75%). Interleukin-23 can be used as a biomarker of airflow obstruction in patients with neutrophilic asthma. Targeting IL-23 has a potential for the development of biologics for precision medicines to treat IL23/IL-17 axis-mediated diseases.

4.8. The treatment effect on markers

This study showed a non-significant association between the EOS count, IgE and immunological markers with treated and non-treated patients as in table (3-8). The current study differs from other studies conducted by (Daikh, Ryan and Schwartz, 2003) and (Yamakawa *et al.*, 2010) whose mentioned the role of montelukast in the reduction of blood eosinophiles, while, the present study were

compatible with a studies achieved by Kopřiva *et al.*, (2004) and Friesen *et al.*, (2009) whose said that there were no significant changes in eosinophil density or eosinophil activation following treatment with montelukast. In addition, the use of a single eosinophil count as a criterion for eligibility for specific therapeutics is insufficient to identify all patients with the potential to benefit (Mathur *et al.*, 2016).

4.9. Receiver operating characteristic curves

The sensitivity and specificity of each of the measured biomarkers were evaluated using the Receiver Operating Characteristic curve. It was found that IL-23 serum level to be of a high significance measuring of diagnosis of pediatric asthma as in table (3-9). This result consistent with a study conducted by Ciprandi *et al.*, (2012) who suggested that serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children. Also, in the same line with a study achieved by Alyasin *et al.*, (2017) who suggested that measurement of IL-23 serum levels as a complementary marker along with clinical criteria for diagnosis of asthma in children less than five years old.

Conclusion and Recommendations

Conclusions:

1- Elevation of IL-23 in contrast to IL-15 and IL-22 in serum of pediatric asthma

2- IL-23 had high sensitivity and specivity in diagnosing pediatric asth,a

3- There was a significant negative correlation between IL-23 level and duration of asthma.

4- The severity of asthma depending on the duration, which was higher in moderate than the mild asthmatic patients.

Recommendations: -

1- Further studies with a larger sample size are required for a better understanding the effect and association of IL-15,IL-22,and IL-23 with severity of asthma disease.

2- Extended studies based on asthma phenotypes (Early wheeze, Allergic asthma, Non allergic, Adolescent & obesity, Late onset and Persist wheezier) are recommended.

3- More studies are required to study the association between the other immunological markers and severity of asthma.

4- Other studies are required for better understanding the relationship between interleukins gene polymorphism and severity of asthmatic children.

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Appendices

Appendix1

participant name		participant number	
Age		gender	☐ female ☐ male
Date of selection	/ / /	file number	

☐ patient

History of eczema	☐ yes	☐ no				
Family history of eczema	☐ yes	☐ no				
Allergic rhinitis	☐ yes	☐ no				
Family history of allergic rhinitis	☐ yes	☐ no				
Allergic conjunctivitis	☐ yes	☐ no				
Exposure to cigarette smoke	☐ yes	☐ no				
pets	☐ cat	☐ dog	☐ birds			
Aggravating factor	☐ upper respiratory	☐ dust	☐ Physical activity			
Treatment	☐ Montelukast	☐ 4	☐ 5	☐ 10		
	☐ Inhaled corticosteroids	☐ Beclomethasone	☐ Budesonide			
		☐ Fluticasone	☐ ICS + LABA			
	☐ Mixed					
Duration						

Severity		Mild		Moderate	Sever

Duration of Asthma				
Asthma Phenotype		Early wheezer asthma < 3y		allergic Asthma
		non- allergic		Adolescent and obesity asthma (female)
		late onset Wheezer		Presist Wheezer

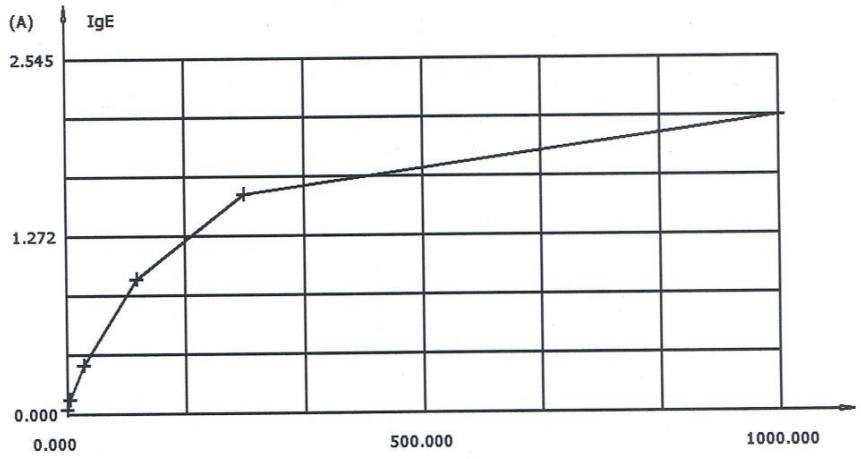
	control

Family history of asthma		yes		no
Family history of allergic rhinitis		yes		no
Family history of eczema		yes		no
Family history of smoking		yes		no

Appendix 2

Standard curve

HumaReader



Program: IgE IgE Caculation: Curve Wavelength(nm): 450/630

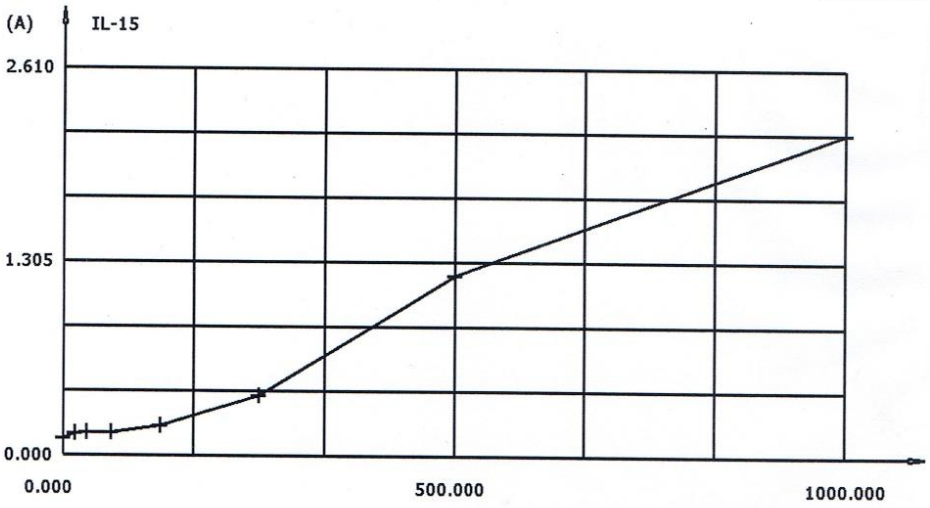
Standard sample	#1	#2	#3	#4	#5	#6
Concentration(IU/ml)	0.000	5.000	25.000	100.000	250.000	1000.000
Abs(A)	0.027	0.099	0.343	0.960	1.568	2.121

Operator: Checker:
Print date: 04/09/2021

A

Standard curve

HumaReader



Program: IL-15 IL-15 Caculation: Curve Wavelength(nm): 450

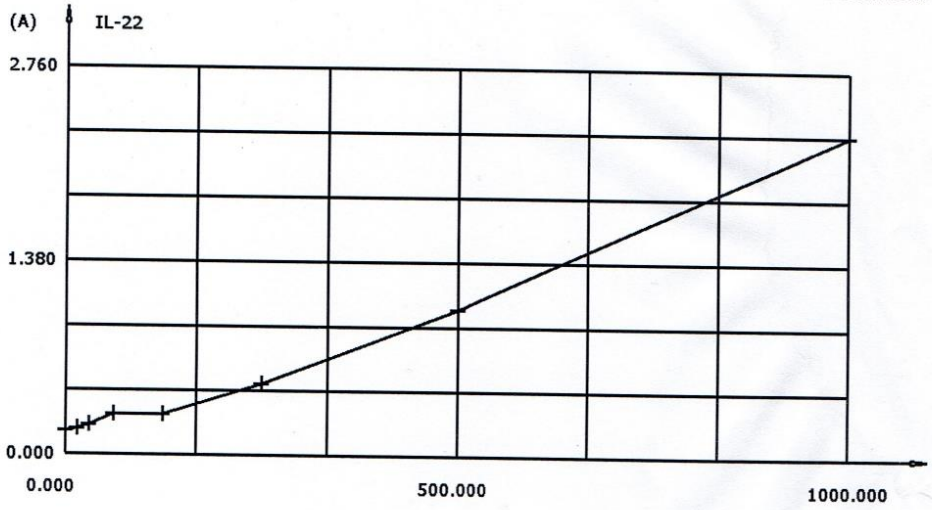
Standard sample	#1	#2	#3	#4	#5	#6	#7	#8
Concentration(pg/ml)	0.000	15.630	31.250	62.500	125.000	250.000	500.000	1000.000
Abs(A)	0.111	0.141	0.148	0.149	0.197	0.402	1.210	2.175

Operator: Checker:
Print date: 04/09/2021

B

Standard curve

HumaReader



Program: IL-22 IL-22 Caculation: Curve Wavelength(nm): 450

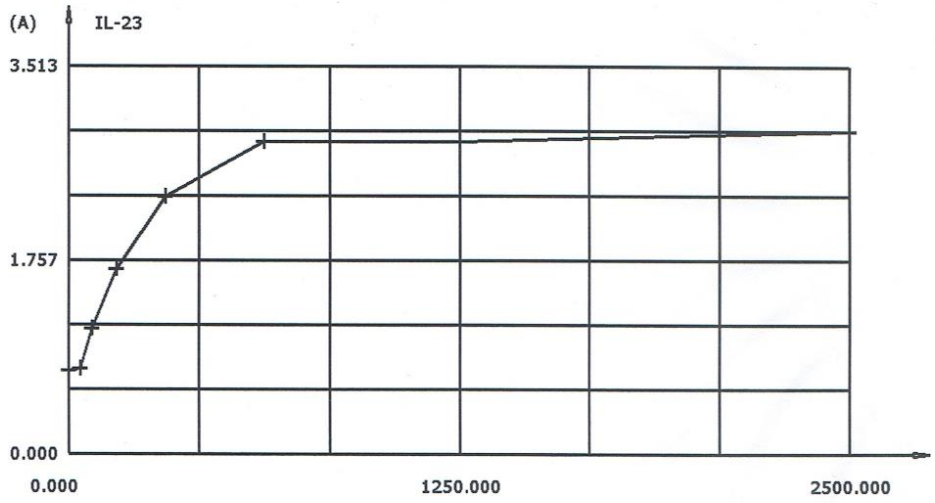
Standard sample	#1	#2	#3	#4	#5	#6	#7	#8
Concentration(pg/ml)	0.000	15.630	31.250	62.500	125.000	250.000	500.000	1000.000
Abs(A)	0.162	0.180	0.207	0.283	0.285	0.506	1.043	2.300

Operator: Checker:
Print date: 04/11/2021

C

Standard curve

HumaReader



Program: IL-23 IL-23 Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6	#7	#8
Concentration(pg/ml)	0.000	39.060	78.130	156.250	312.500	625.000	1250.000	2500.000
Abs(A)	0.747	0.770	1.128	1.670	2.329	2.825	2.830	2.928

Operator: Checker:
Print date: 04/11/2021

D

الخلاصة

الربو هو مرض التهابي مزمن شائع للشعب الهوائية في الرئتين ، ويعتبر مشكلة صحية عالمية كبرى ، حيث يصيب على الاقل من3.5 الى 20 % من سكان العالم . يصيب الربو الناس في جميع الاعمار ولكنه في الغالب يبدأ في مرحلة الطفولة . الاستجابة الالتهابية تنشأ عن طريق غلوبولين الحساسية المناعي E الذي ينتج خلال أستثارة الحساسية ، والذي سيرتبط مع الخلايا البدينة عند اعادة التعرض للمحسس .

الكلوبيولين المناعي E يلعب دوراً فعال في امراضية الربو التحسسي. يعتقد بان الانترلوكين 15 اساسي للحفاظ على فرط استجابة مجرى الهواء الناجم عن مسببات الحساسية ، بينما الانترلوكين 22 يلعب دوراً اساسيا في عملية التحسس من المستضد في نماذج فئران التجارب المصابة بالربو . كذلك يعتقد بان الانترلوكين المصلي 23 يمكن ان يكون مؤشر لضعف وظائف الشعب الهوائية في الأطفال المصابين بالربو التحسسي.

نوع الدراسة الحالية هي دراسة الحالات والسيطرة حيث شملت 60 طفل مصاب بالربو (35 منهم ذكور و25 اناث) كمجموعة مرضى تتراوح اعمارهم من 4 شهر الى 14 سنة حضروا الى العيادة الاستشارية لمرض الربو في مستشفى الاطفال التعليمي للفترة من شهر تشرين الثاني لسنة 2020 ولغاية شهر اذار لسنة 2021 ، و60 طفل غير مصاب بالربو (32 منهم ذكور و 28 اناث) اعمارهم تطابق مجموعة المرضى تم اتخاذهم كمجموعة سيطرة.

تم جمع الدم من كل مشترك ، مع الملاحظة ان المصل استخدم لتحديد كمية ومستوى الكلوبيولين المناعي E و الانترلوكينات 15 و 22 و23 لكل عينة بأستخدام تقنية الاليزا ، بينما استخدم الدم الكامل لتحديد عدد كريات الدم الحمضة بأستخدام جهاز سيمكس XN-350 خماسي الابعاد الالي.

اظهرت الدراسة وجود علاقة معنوية عالية بين مستوى الكلوبيولين المناعي نوع E و الانترلوكين 23 في المصل مع مرضى الربو (P=0.005, P=0.008) بالتعاقب. كذلك، اظهرت الدراسة فرق معنوي بين الربو الخفيف والمعتدل فيما يخص تفاقم المرض بالتهاب الجهاز التنفسي العلوي . (P = 0.031)بالاضافة الى ذلك ، هناك اختلاف معنوي (P = 0.049) بخصوص العلاج بين مرضى الربو الخفيف والمعتدل.

فيما يتعلق بالعلاقة بين المؤشرات المدروسة ومدة الربو ، كان هناك ارتباط سلبي معنوي (p = 0.030)بين مستوى الانترلوكين 23 في المصل ومدة الربو.

أظهرت الدراسة الحالية فرقاً معنوياً (P = 0.046) في شدة الربو تبعاً لمدة المرض ، حيث كانت الشدة أعلى في الربو المعتدل من مرضى الربو الخفيف بمتوسط (36.70) و (17.86) على التوالي. كان هناك فرق معنوي (P = 0.004) في عدد كريات الدم الحمضة وفقًا لكل نوع من الربو في الدراسة الحالية ؛ حيث كانت الأعلى في الربو التحسسي بمتوسط.(1.2518)

أظهرت نتائج هذه الدراسة ارتفاع مستوى الانترلوكين 23 في مصل مجموعة المرضى مقارنة مع مجموعة السيطرة ، لذلك ، قد يكون للانترلوكين 23 دور مهم في تشخيص الأطفال المصابين بالربو الحاد.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية الطب
فرع الاحياء المجهرية

علاقة أنترلوكينات المصل 15 و22 و23 مع شدة ربو الاطفال

رسالة مقدمة الى

مجلس كلية الطب - جامعة كربلاء كجزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية الطبية

مقدمة من قبل

بنين احمد جبار الطرفي

بكلوريوس علوم حياة / جامعة كربلاء – كلية العلوم 2016

بأشراف

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1443هـ 2022 م