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NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

# THE ROLE OF HYPOGAMMAGLOBULINEMIA IN CHILDHOOD ASTHMA

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PhD in IMMUNOLOGY AND ALLERGY

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THESIS ADVISOR

PROF.DR. NERİN BAHÇECİLER ÖNDER

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## ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Prof. Dr. Nerin BAHÇECİLER ÖNDER for the continuous support of my PhD study and related research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study.

Besides my advisor, I would like to thank the rest of my thesis committee: Prof.Dr. Arzu BABAYiĞiT, Prof. Dr. Turgut İMiR, Prof. Dr. Günnur DENiZ, Assoc. Prof. Dr.Nilüfer GALİP ÇELİK, Assoc. Prof. Dr. Murat UNCU, Asst.Prof. Dr. Umut GAZi, for their support, insightful comments and encouragement during my PhD study.

My sincere thanks also goes to Asst.Prof. Dr. Burçin Şanlıdağ. Without her precious support it would not be possible to conduct this research.

Many thanks to Asst.Prof. Dr. Özgür Tosun for his support on statistical analysis.

I would also like to thank my lab mates in Near East University Hospital Biochemistry Laboratory.

I am grateful to my parents, Dr. Sevgi ÖKSÜZ, Dr. Asım YÜRÜKER and to my husband Dr. Enver KNEEBONE for their eternal support during my PhD study.

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## Abstract

**YÜRÜKER Ö. The Role of Hypogammaglobulinemia in Childhood Asthma. Near East University Institute of Health Sciences, Immunology and Allergy, PhD Thesis, 2018.**

The relationship between hypogammaglobulinemia and wheezing in childhood have been previously revealed.

The aim of this study is to investigate comorbid immune deficiency in unresponsive asthmatic children, characterize the type of immune deficiency and study any possible effect of immune deficiency treatment on the applied standard guideline asthma treatment.

This was a retrospective study conducted between January 2012 and December 2014. The medical records of 286 children whose serum Ig levels had been analysed before were collected. Among those children, 125 (M:F,79:46, mean age:41.3 ±25.2 months) enrolled as they had uncontrolled moderate to persistent asthma. Those 125 children were categorized as only asthma and asthma+ hypogammaglobulinemia (HGG) groups depending on their immunoglobulin (Ig) concentrations.

Seventy six of 125 children (60.8%) had comorbid hypogammaglobulinemia. Atopy was higher in Asthma+ HGG group (p=0.044). The most frequent comorbid HGG were transient hypogammaglobulinemia of infancy (THGI) (46.1%) and IgG subclass deficiency (32.9%). Although the comparison of percentage use of ICSs was not significantly different between the two groups at the initial evaluation, the dose of ICSs significantly decreased only in asthma+ HGG group (p=0.017).

The majority of asthmatic children having symptoms despite of appropriate guideline based treatment, may have comorbid immunological abnormality. Presented data demonstrated the necessity of immunological evaluation in uncontrolled asthmatic children to prevent long term side effects of high dose ICSs, reduce the frequency and severity of asthma symptoms, and improve the quality of life.


**Keywords:** Childhood Asthma, Hypogammaglobulinemia, wheezing, immunoglobulin levels.

## Abbreviations

BHR: Bronchial Hyperreactivity

CD: Cluster of Differentiation

CVID: Common Variable Immune Deficiency

g : Gravity

HGG: Hypogammaglobulinemia

ICS: Inhaled Corticodteroid

Ig: Immunoglobulin

ml: milliliter

PHGI: Protracted Hypogammaglobulinemia of Infancy

PID: Primary Immune Deficiency

RAST: Radioallergosorbent Test

SD: Standard Deviation

SPT: Skin Prick Test

THGI: Transient Hypogammaglobulinemia of Infancy

µl: microliter

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# 1. THE PROJECT OBJECTIVE

Asthma is an immunological disease which is characterized by persistent inflammation of the airways, bronchial hyperreactivity (BHR), recurrent wheezing, cough and shortness of breath (Martinez & Vercelli, 2013) It is common in childhood with different phenotypes that are characterized by persistence or remittance of wheezing (transient early wheeze, non-atopic wheeze and atopic-asthma) (Stein & Martinez,2004; Sly et al,2008)

When children have partial or no response to the asthma treatment, the asthma guidelines usually recommend to accept the patient as a more severe case and to increase the dose of ICSs or add another asthma treatment (GINA guidelines, 2015). The follow-up protocol of the Paediatric Allergy and Immunology Division of the Near East University Hospital, asthmatic children with recurrent wheezing, cough and croup who have partially responded or have not responded to the standard asthma medications and environmental precautions, are further evaluated in order to rule out comorbidities including gastroesophageal reflux, sinusitis, cystic fibrosis, and immune deficiency diseases before accepting the patient as a more severe asthma case. Following the rule out of gastroesophageal reflux and sinusitis, a sweat test for cystic fibrosis is performed if needed. Then, evaluation of the immune system is performed including measurement of serum Ig and Ig subclass levels. Further tests such as determination of the lymphocyte subset percentages and specific antibody responses are performed if indicated by the clinician.

In this project, children (i) with recurrent wheezing, cough and/or croup who were unable to decrease inhaled steroid dose for control of symptoms (partially controlled) according to guideline recommended treatment (GINA guidelines, 2015), (ii) who were investigated for immunodeficiency (iii) who had a follow up period more than 6 months (iv) who did not receive allergen-specific immunotherapy were included. The demographic, clinical and laboratory findings

of the children included in the study were collected from the hospitals database programme retrospectively in order to find out whether the underlying cause of uncontrolled childhood asthma was due to accompanying hypogammaglobulinemia.

It was previously reported that, significant number of children with recurrent wheezing had comorbid hypogammaglobulinemia (HGG) highlighting the presence of a causal relationship between HGG and childhood wheezing in the general population (Barıs et al,2011; Alvaro et al, 2013; Szczawinska-Poplonyk,2012; Oner et al,2000; Karaman et al,1999).In one study, a 66.6% of $IgG_3$ deficiency had been reported among recurrent wheezers (Alvaro et al, 2013), while another study had reported a 36% of HGG among children with asthma (Barıs et al,2011). Immunoglobulin (Ig) levels normalize in some children at earlier age, while some of them retain persistent low Ig levels (Ozen et al, 2010). However, little is known about the importance of treating the comorbid HGG alongside the asthmatic symptoms.

One of the clinical observations is that a significant number of asthmatic children have hypogammaglobulinemia or IgG subgroup deficiencies and when those underlying causes are treated, asthma symptoms are controlled without increasing dose or by adding variety of asthma medications. Based on this clinical observation we hypothesized that hypogammaglobulinemia can be an important causative factor for the development of childhood asthma.

The aim of this study is to investigate comorbid immune deficiency in asthmatic children who are unresponsive/partially responsive to the standard guideline treatment, characterize the type of immune deficiency and studying any possible effect of immune deficiency treatment on the applied standard guideline asthma treatment.

# 2. INTRODUCTION

## 2.1. The Immune System

The word ‘immunity’ (Latin *immunitas*, freedom from) defines a defence mechanism that includes various biological structures within organism that protect against pathogens and other foreign bodies that cause diseases. The immune system is highly discriminatory and essential for survival. (Delves et al, 2006)

Immune system is further classified into two subgroups; the innate immune system and adaptive immune system. All cells that involve in the protection of the body originate from the bone marrow, where many of them also mature. Then they migrate to peripheral tissues through the lymphatic system. During this migration, they receive signals via their receptors that contribute their activation, proliferation and survival.

The innate immune system, nonspecific immune system, provides immediate defence against infections. It is an important subsystem of the overall immune system whose cells recognize and respond to pathogens in a generic way, but does not confer long-lasting or protective immunity to the host. If the microorganism penetrates the body two main defence mechanisms come into play; proteolytic enzyme activation for destruction of bacteria and phagocytosis, engulfment along with digestion of microorganisms which is performed by specialised cells called phagocytes for example macrophages. Macrophages act also as antigen presenting cells (APC) that lead the activation of the second branch of the immune system, the adaptive immune system.

The adaptive immune system, acquired immune or the specific immune system, is a subsystem of the immune system that is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth. Unlike innate immune system, adaptive immune system can also generate immunological memory after an initial response to a specific pathogen and provides long lasting protection. Adaptive system includes both humoral

immunity components represented by B-lymphocytes and cell-mediated immunity components maintained by T- lymphocytes.

Humoral immune system plays a crucial role with its antibodies that are produced by B lymphocytes for protection against variety of microorganisms that cause infection to the host. Defects in development, selection and function of B cells results in the clinical manifestations of antibody deficiencies. Besides recurrent infections, it has been reported that asthma and other allergic diseases are also frequent inpatients with antibody deficiencies. (Delves et al, 2006; Male et al, 2006)

Asthma is an immunological disease which is characterized by persistent inflammation of the airways, bronchial hyperreactivity (BHR), recurrent wheezing, cough and shortness of breath. It is common in childhood with different phenotypes (Stein & Martinez, 2004.)

Although there have been several reports on the relationship between various immunoglobulin deficiencies and wheezing in childhood, the importance of this association is not yet clear (Barıs et al,2011; Alvaro et al, 2013; Szczawinska-Poplonyk,2012; Oner et al,2000; Karaman et al,1999).

### 2.2. The Humoral Immune Response

The adaptive immune response is a sophisticated system that enables a specific reaction to a huge variety of foreign molecules that are non-self. It has two distinct arms by which it exerts its function. These are; the cellular immune response which is T-cell mediated and the humoral immune response which is antibody mediated. For the action of these systems the foreign antigens are needed to be recognised by T-cells and B-cells through their highly variable receptor molecules called ‘T cell receptor (TCR)’ and ‘B cell receptor (BCR)’ respectively. (Janeway *et al*. 2005)

Many of the bacteria that cause infections multiply in extracellular spaces of the body. These spaces are protected by humoral immune response in which antibodies are produced by B-cells. When B-cells become activated due to presence of the foreign antigen, they develop into plasma cells. The latter cells produce specific antibodies to that specific antigen. The function of the antibodies are to destruct extracellular microorganisms and to prevent the spread of intracellular infections. Antibodies carry out this function by; neutralization of the

antigen by binding to it, facilitating the uptake of pathogens by phagocytic cells and activating the complement cascade.

### 2.2.1. Immunoglobulins (Antibodies)

Immunoglobulins are family of glycoproteins that function either as membrane-bound form on the B-cells or as soluble form found in serum or tissue fluids secreted from the plasma cells. There are five distinct types of immunoglobulins; IgM, IgG, IgD, IgA and IgE. IgG is further divided into four subclasses, IgG1, IgG2, IgG3, IgG4 and IgA into two subclasses; IgA1 and IgA2. These molecules differ in size, charge, amino acid sequence and carbohydrate content.

X-ray crystallography revealed that the basic structure of immunoglobulin molecules consists of two light polypeptide units and two heavy polypeptide chains. The light chains (25 kDa) are bound to heavy chains (55 kDa) by interchain disulphide bridges and multiple non-covalent interactions. The heavy chains are bounded to each other in the same way. Each segment of approximately 110 amino acids that are folded to compact domain stabilised by covalent intrachain disulphide bonds. The light chains are found in two distinct forms; kappa (κ) and lambda (λ). Either of the light chain type may combine with any heavy chain types, but in any individual immunoglobulin molecule both light or heavy chains are of the same type.

Within variable regions of both heavy and light chains, there are some polypeptide segments called hypervariable regions or complementary determining regions (CDRs) that are involved in antigen binding by creating an interaction site which is complementary in shape, charge and hydrophobicity to the epitope it binds. The structure of the heavy chain determines the class and the subclass of the immunoglobulin. (Male *et al*. 2006).

B-cells develop in the bone marrow (BM) from hematopoietic stem cells. These cells progress through an irreversible cascade of differentiation steps in a complex process influenced by various microchemical factors. (Ballow, 2002)

Two main phases are present in B-cell development: antigen independent and antigen dependent phase. In antigen independent phase, diverse repertoire of antigen-specific B cells develops in the bone marrow. In antigen dependent phase, B cells are stimulated by antigen and undergo activation and clonal expansion in peripheral lymphoid tissues. During clonal expansion, further

diversity and specificity according to the antigen develops through somatic hypermutation.

The generation of antibody is one of the most important parts of the immune response and is the basis for the vast majority of successful vaccination strategies. Antibody is produced by rare populations of terminally differentiated B cells known as plasma cells. The plasma cell formation is associated with marked alterations in the morphology, gene expression profile and lifespan of the differentiated antibody secreting cells (ASCs) compared with their B cell predecessors.

### 2.3. Primary Immune Deficiency Disorders: Antibody Deficiency

Primary immunodeficiency disorders are defined according to which part of the body's immune system is missing or not functioning properly. These disorders are caused by hereditary genetic defects and not by secondary factors i.e. caused by other diseases, depending on treatment or environmental toxins. Primary immunodeficiencies are mainly divided into specific (T and B cells) and innate (complements and neutrophils) immune system deficiencies. These disorders are characterized by recurrent or persistent infections. The nature of infection usually gives clue about the disorder. (Adelman *et al*. 2012)

Unlike T-cell deficiencies, patients with antibody deficiencies do not suffer from infections until 7-9 months after birth as maternal antibodies, mainly IgG, that have passed to foetus during pregnancy via placenta protects the child. After about 9 months these transplacental antibody titres decrease to below protective levels. (MOISE, A.et al, 2010). Immunoglobulins are produced by B-cells and are the main mediators of humoral immune system. B-cell defects result in low levels of immunoglobulins in the blood circulation and latter affect the body's immune response to infections. Primary hypogammaglobulinemias are usually characterized by recurrent or persistent infections. Individuals with antibody deficiencies usually get infected with encapsulated bacteria e.g. *Streptococcus pneumonia.* Major B-cell immunodeficiences are; transient hypogammaglobulinemia of infancy, selective IgM deficiency, Bruton agammaglobulinemia, selective IgA deficiency, IgG subclass deficiency and common variable immunodeficiency (CVID). Most of the patients can lead

normal lives by taking prophylactic antibiotics. However for severe infections, subcutaneous immune serum globulin or intravenous immune serum globulin (IVIG) treatments are needed to be given (Ballow, 2002).

### 2.3.1. Transient hypogammaglobulinemia of Infancy (THI)

A newly born baby has significant amount of immunoglobulin G (IgG) that have passed from the maternal placenta during the last trimester of pregnancy. Infants normally start to synthesize IgG only after 2-4 months of age and IgA, IgM even later. From that time immunoglobulin (Ig) levels in the blood circulation decreases and declines at about 6 months of age. Some infants have an abnormal delay in the onset of Ig synthesis and those infants have significantly lower Ig levels than infants at the same age. This prolonged synthesis is usually recovered at the second or third year of life. THI may be due to the presence of genetic variation of the family, abnormal T-lymphocytes that fail to stimulate antibody production of B-cells, an unbalanced cytokine production or an abnormal B-cell development.

Affected patients show various clinical signs including recurrent upper respiratory tract infections such as sinusitis, less commonly pneumonia, allergy and gastrointestinal difficulties. IVIG therapy is not usually necessary if the infection is not that severe and the child is responding to the prophylactic antibiotic treatment. These patients have low IgG and IgA levels but usually normal or high IgM levels. Most of the children have normal antibody response to vaccines including tetanus, hepatitis A and B, measles-mumps- rubella (MMR). Tetanus is a potent vaccine and lack of antibody response suggests a more serious defect of the immune system. Most of the children with THI starts to develop appropriate levels of Ig by the age of three. However, for some of the children, the condition may persist until the age of five having protracted hypogammaglobulinemia. (Ballow, 2002)

### 2.3.2. IgG Subclass Deficiencies

In IgG subclass deficiencies, the IgG subclass level is more than 2 standard deviation (SD) below the normal mean of age. The IgG subclass level is age-specific, therefore, the level of IgG needs to be checked according to the specific age ranges. Detection of low or absent subclasses do not necessarily correlate with the clinical symptoms.

B and T cell numbers are usually normal and IgG levels are compatible with the subclass deficiency. Most of the time, IgG deficiency (IgG2 and IgG4) is associated with IgA deficiency. Therefore, these patients do not respond to polysaccharide vaccines against *Pneumococcal penumonia* or *Haemophilus influenza*. (Moise et al,2010)

IgG2 and IgG4 deficiencies generally increase the susceptibility to recurrent infections of upper and lower respiratory tracts as IgG2 plays a role in elimination of polysaccharide agents. Asthma and sinusitis also occurs mostly due to IgG3 deficiency.

Only symptomatic patients need to be treated. If recurrent infections persist, then regular prophylactic antibiotics can be given as a first step of the treatment. If the infections cannot be controlled then the treatment can be supported by IVIG therapy. (Spickett, 2013)

### 2.3.3. Selective IgA Deficiency

First selective IgA deficiency case was described in 1962. IgA deficiency is the most common disorder as 1:400-800 people show symptoms. Although B-cell levels are normal, IgA is not secreted from plasmocytes. It seems that there is a lack of B-lymphocyte response to IL4, IL6, IL7 or IL10. (Moise et al, 2010)

Patients may suffer allergic diseases including asthma, food allergies and intolerances. Also, range of autoantibodies are increased and organ specific autoimmune diseases may occur. People with total IgA deficiency develop anti-IgA antibodies. And in the case of blood transfusion, these patients may develop adverse reactions. IgA deficiency can be associated with other immune

deficiencies especially IgG subclass deficiencies and lack of response to pneumococcal vaccine. Consequently, some patients develop common variable hypogammaglobulinemia (CVID). (Moise et al, 2010)

In selective IgA deficiencies the serum IgA level is less than 0.05g/dL which is undetectable, however total IgG and IgM levels are usually normal. Sometimes, IgG2 and IgG4 levels decrease. In the absence of IgA, there is an evidence that IgM and IgG may substitute as secretory immunoglobulins on mucosal surfaces. (Spickett, 2013)

The treatment is via prophylactic antibiotics for the prevention of infections. IVIG therapy may be needed for persistent infections. (Moise et al, 2010)

### 2.3.4. Common Variable Immunodeficiency (CVID)

CVID is the second most common primary immunodeficiency disease affecting both sexes equally. Disorder is characterized by low titres of serum immunoglobulin levels due to inability of B-lymphocytes to differentiate into plasma cells and therefore affected patients are not able to produce not antibodies. This increases susceptibility to infections. The incidence rate is 1:10.000-50.000 depending on particular race. (Moise et al, 2010)

CVID is a hereditary disorder and usually occurs within the same family. Individuals who have an IgA deficiency disorder may subsequently develop CVID. The major CVID/IgA deficiency susceptibility gene, IGAD1 maps to HLA-DQ/DR region of MHC. (Adelman *et al*. 2012)

Most patients have an antigen-presenting dysfunction as there is a defect in T-cell priming by antigen.

The deficiency may present at any age. The patients suffer from chronic respiratory diseases; sinusitis, otitis, pneumonia. As IgG production is low, encapsulated bacteria are the common pathogens. Autoimmune diseases such as thrombocytopenia, haemolytic anaemia and thyroiditis may be present. Patients may also present infectious diarrhoea. (Moise et al, 2010)

Treatment consists of intravenous and subcutaneous immunoglobulin administration every 2-4 weeks in order to maintain normal antibody titres. However for gastrointestinal problems, corticosteroids or other immunosuppressant drug usage may be needed. (Spickett, 2013)

### 2.3.5. Selective IgM Deficiency

IgM deficiency is a rare disease as only about 300 cases have been described in the literature. Selective IgM deficiency is observed in both children and adults with no gender bias. The most common clinical manifestation of selective IgM deficiency is infections with extracellular and intracellular bacteria, viruses, and fungi. Allergic reactions are the second commonest presentations of selective IgM deficiency. In children with selective IgM deficiencies, infectious manifestations are more common than allergic and autoimmune diseases. Malignancies are infrequent. İn adults with selective IgM deficiencies, recurrent upper and lower respiratory tract infections are common. Allergic and autoimmune diseases are also frequently present. İn some cases, Selective IgM deficiency is associated with 22q11.2 chromosome deletion and fewer cases of familial selective IgM deficiency have been reported.

The treatment of IgM deficiency is with the administration of IVIG therapy. (Moise et al, 2010)

### 2.3.6. Bruton (X-linked) agammaglobulinemia

Bruton (X-linked) agammaglobulinemia is a genetic disorder due to the mutations (either deletion or point mutation) on the bruton tyrosine kinase (Btk*)* gene on the X chromosome (location Xq21.3-22).

*The* Btk gene codes for Bruton tyrosine Kinase which plays an important role in B-cell maturation. Defects in gene coding, prevents the maturation of B-cells from Pro-B cell stage to Pre-B-cells. Therefore patients have low Ig level titres as their plasmocytes do not properly develop.

This is the first immunodeficiency to be described in 1952. The disease affects 1 in 10000-200000 males. Females do no to show any clinical symptoms as they are only carriers. Clinical symptoms start after 6 months as the level of placental antibodies start to decrease. Presentation of this disorder is usually includes recurrent infections in the lungs and ears, caused by pathogens such as *Haemophilus influenza*, Streptococcus *pneumococci* (upper and lower respiratory tract), *Nisseria meningitidis* (meningitis), *Giardia*, *salmonella ssp* infections of the gut.

Laboratory analysis show that, the IgG titres are low below the value of 100mg/dL.

B-cell and T-cell count can be confirmed with flow cytometry analysis. B-cells are at low levels or absent and T-cells are usually at normal levels. BTK protein is absent because of the Btk gene mutation. There could be some complications related to delay in diagnosis of the disease such as chronic meningoencephalitis, ureaplasma/mycoplasma arthritis and haemophilus conjunctivitis. (Spickett, 2013)

There is no cure for the disease. For the treatment, IVIG therapy should be given at the earliest opportunity (200-600mg/kg/month) to maintain the IgG levels of 500-800mg/dl with intervals of 2-3 weeks. The Titre of Ig level should be checked regularly.

Together with the IVIG therapy, prophylactic antibiotic therapy and bronchodilators can be given for the upper and lower respiratory tract infections. İf the lung damage has already occurred, physiotherapy and postural drainage may be needed. High resolution computed tomography (CT) scans can be useful for detecting the subclinical bronchiectasis. Multivitamins and additional nutritional supplements are also recommended. (Spickett, 2013; Moise et al, 2010)

### 2.4. Asthma

Asthma is one of the most widespread chronic diseases in developed countries characterized by bronchospasm. It is an inflammatory disorder of the airways in which many immune cells and cellular elements play specific roles. The chronic inflammation is associated with the airway responsiveness that leads to recurrent periods of wheezing, shortness of breath, chest tightness and coughing. (Lambrecht & Hammad, 2015.)

Asthma is thought to be caused by a combination of genetic and environmental factors. Children whose parents have asthma tend to have a higher risk of having asthma themselves. Asthma does not follow the Mendelian gene pattern as it involves multiple genes.

Besides genetic factors, viral infections and various environmental factors such as aeroallergens including pollens, animal dander and house dust-mites along

with air pollutants which are irritants like sulphites are potent inducers of asthma symptoms. Exposure to tobacco smoke during pregnancy and after the delivery of the baby, increases the risk of developing asthma-like symptoms such as wheezing in the first year of life. Indoor allergens increase the risk factor of developing wheezing independently.

Asthma is clinically classified according to the frequency of the symptoms, forced expiratory volume in one second (FEV1) and peak expiratory flow rate.

Allergy is an immune-mediated response to normally harmless environmental substances. Allergens can cause various allergic diseases including asthma and allergic rhinitis. In atopic asthma, the serum IgE levels are elevated, peripheral blood eosinophilia count increases and the patients have high allergen-specific IgE levels which can be either measured in serum samples or by performing a skin prick test with specific allergens.

Atopy is a genetic disposition to develop an allergic reaction. In atopic asthma candidate genes have been identified by genome wide screens from the cytokine gene cluster on chromosome 5 and MHC on chromosome 6 including GSTM1,IL10,SPINK5, CTLA4, ADAM 33, IL4R. (Ober and Yao, 2011)

Although, the majority of patients have extrinsic (atopic) asthma, some patients are non- atopic (intrinsic or idiopathic) asthma which doesn't involve allergens and are mainly caused by viral infections of the respiratory tract along with inhaled air pollutants such as sulphur dioxide, ozone and nitrogen dioxide. Intrinsic asthma patients show negative skin prick tests and lack of specific IgE titres. Patients with intrinsic asthma tend to have onset of symptoms later in life and are more commonly female. Non-atopic asthma is more severe than allergic asthma and is more difficult to treat.

There is no specific test for the diagnosis of asthma. Therefore, diagnosis is generally based on clinical observation, atopic disease history of the patient and family, physical examination and various laboratory tests.

Immunological features of asthma include activation of mast cells which causes the synthesis of cytokines; IL-3, IL-4 and IL-5 that are involved in the stimulation of eosinophils. The Th1:Th2 balance favours towards the Th2-mediated reaction. Level of Th2 cytokines (IL-4 and IL-5) increase, leading to further IgE production, downregulating the Th1 responses.(Figure 2) (Lambrecht and Hammad, 2015)

Childhood is the period of greatest incidence of asthma. The most common symptoms of asthma are recurrent wheezing and cough. Approximately 80% of patients produce sputum from the lungs by coughing. Wheezing results from the turbulent flow through constricted airways. Cough is caused by the stimulation of the sensory nerves resulting from the bronchoconstriction and mucosal depositions. As the asthma patients have increased bronchial reactivity, anything that irritates the airway has a potential to cause bronchoconstriction. Patients usually report that their asthma conditions worsen during respiratory infections. Symptoms of asthma are usually worse at night and early in the morning due to hormonal changes.

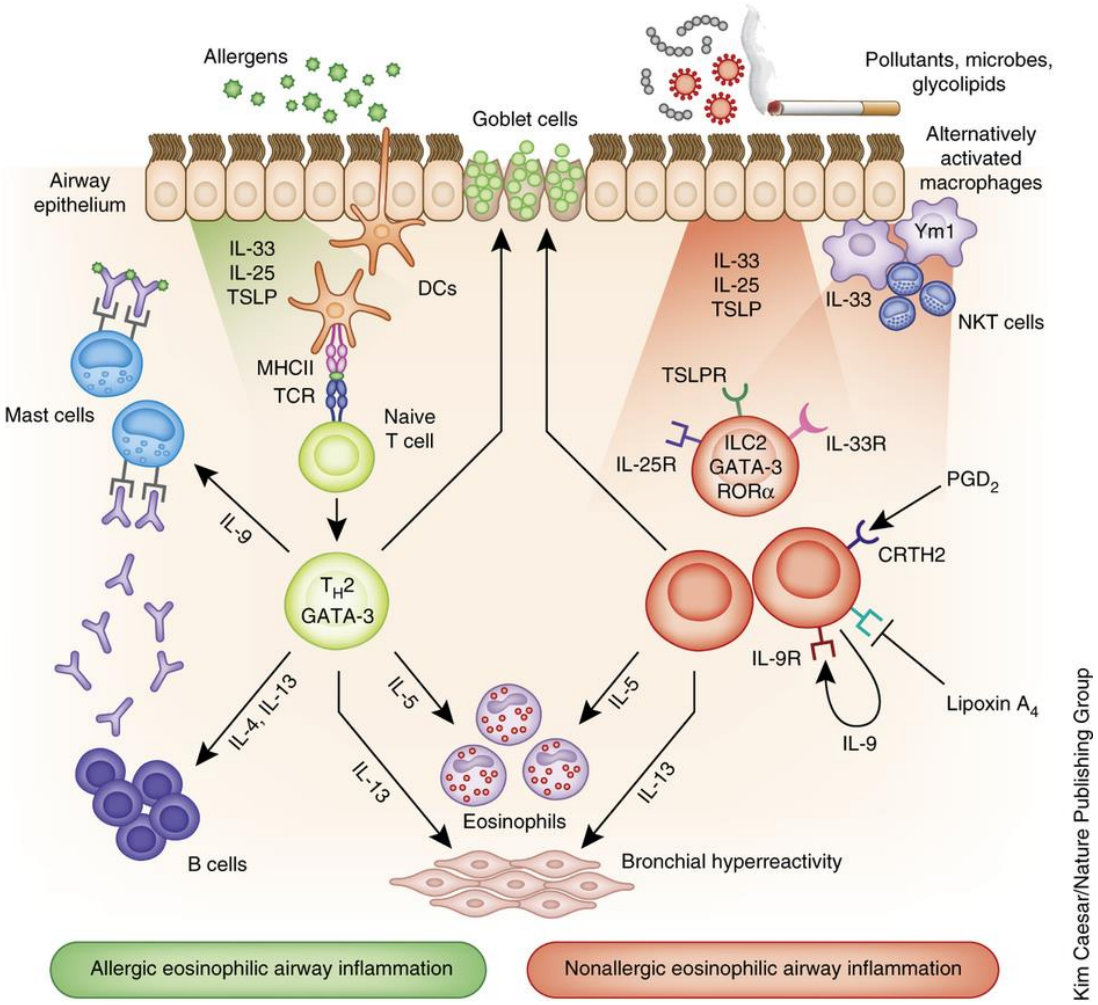


**Figure 1:** Relative roles of Th2 cells and Type 2 innate Lymphoid (ILC2) cells in two forms of eosinophilic asthma. In atopic asthma (left), eosinophilic airway inflammation and bronchial hyperreactivity (BHR) are driven by adaptive Th2 cells that are stimulated by DCs to produce IL-5, IL-13 and IL-4, the latter driving IgE synthesis. In nonatopic or intrinsic asthma (right), which is not dependent on adaptive immunity, ILC2 cells produce IL-5 and IL-13 and thus cause eosinophilia and BHR. As there is no specific allergen involved and as ILC2 cells produce little IL-4, there is no associated IgE response from B cells. MHCII for MHC class II; TSLPR, receptor for TSLP; NKT cells, natural killer T cells. (Bart N Lambrecht and Hamida Hammad, 2015)

## 2.5. Asthma Phenotypes in Childhood

Asthma is phenotypically heterogeneous and different factors affect the phenotype of asthma determining both its development and severity. These factors include genetics, perinatal exposures, gender, atopy, respiratory infections and other environmental factors. Understanding of different phenotypes of asthma is important for finding new strategies for primary prevention of the disease. (Martinez, 2002).The early identification of children's atopic status is also essential for setting the basis of future preventative strategies as the atopy plays an important role in the development of persistent asthma. The persistent asthma describes the disease that persists from early childhood into adulthood (Sly et al, 2008). Wheezing is the main clinical expression of asthma and is associated with the airflow restriction through narrowed airways causing a characteristic 'whistling' sound. However, wheezing is not a specific symptom for asthma as anything that decreases the diameter of the airway walls could lead to wheezing. The phenotypes of asthma are characterized by persistence or remittance of wheezing. Three epidemiologically distinct phenotypes have been identified in childhood based on specific features: Transient early wheeze, non-atopic wheeze and atopic-asthma (Stein and Martinez, 2004). When a young child with wheezing below the age of 5 is evaluated, clinicians generally determine whether the symptoms represent transient, viral induced wheezing or whether the sufficient risk factors are present to suspect that the child may experience recurrent wheezing and develop asthma.

### 2.5.1. Transient Early Wheezing

In the transient early wheezing, children have recurrent wheezing between the ages of 3 and 5 but rarely afterwards. This phenotype is not usually related with family history of asthma or atopy. İt is thought to be associated with reduced lung function before any lower respiratory illness. This reduced lung function improves with growth, but it still remains lower than that in children who had never wheezed with normal lung functions. Several studies have suggested that, transient early wheezing is related to changes in mechanical pulmonary characteristics such as airway resistance or dynamic compliance. Risk factors for transient early wheezing in children include; prematurity, being exposed to siblings or other children at day care centres, maternal smoking during pregnancy and postnatal exposure to tobacco smoke (Stein and Martinez, 2004)

### 2.5.2. Non-atopic Wheezing

It is a persistent wheezing that is associated with lower respiratory illnesses (LRI) mainly caused by viral infections (%90) such as rhinoviruses, respiratory syncytial virus and parainfluenza viruses (Stein and Martinez, 2004). It is common during childhood especially in the first 3 years of life. İt occurs due to inflammation and oedema of the airway epithelium which decreases the airway diameter. Non-atopic wheezers are different from transient early wheezers as they have normal lung functions at the beginning but end up with enhanced airway reactivity and slightly lower lung functions later in their childhood (Sly et al., 2008).

### 2.5.3. Atopic Wheezing/ Asthma

Persistent wheezing mostly starts before the age of 6 and 60% of the patients are allergic to at least one local aeroallergen by that age (Martinez, 2002). For persistent wheezers, allergic sensitization increases the risk of chronic airway inflammation and the prevalence of respiratory symptoms. Several studies have shown that recurrent wheezing in childhood and asthma have strong association with high Ig E levels and allergic sensitisation to specific allergens. (Stein and Martinez, 2004). In the study performed by Ulrik and Baker, children between the age of 7 and 17 years were enrolled. After 6 years, children who demonstrated either persistent asthma with atopy or persistent asthma with new onset sensitization to house dust mite (HDM*)*, had reduced lung functions when compared to those who remained unsensitised. Loss of lung function was greater in the persistent atopic children compared to those with new onset atopy (Sly *et al*., 2008). All of the allergic asthmatic children continued to have symptoms during adolescence. Children whose asthma initiated before the age of 7, had been sensitized early in their lifetime which persisted when compared to children who did not have asthma until that age. These results suggest that a genetic predisposition to develop sensitization to certain aero-allergens is associated with early asthma symptoms (Stein and Martinez, 2004).

## 2.6. Recommendations for Primary Prevention of Childhood Asthma

Most important risk factors for the development of asthma are genetic predisposition and environmental factors. As, currently it is not possible to alter genetic background, manipulation of the known environmental risk factors is still the only available approach for the prevention of the disease.

Currently, there is no established primary preventative strategy for the development of asthma or airflow limitation in asthmatic patients. (Martinez and Vercelli, 2013)

However, there are specific recommendations aimed at reducing the risk of a child developing asthma. These recommendations suggest that, children should

not be exposed to tobacco smoke during pregnancy or after birth, exposure to allergens and pollutants should be minimized and the use of broad-spectrum antibiotics in the first year of life should be discouraged. Breast-feeding is also advised for further protection (Reddel *et al*, 2015).

### 2.7. Asthma Treatments

Asthma treatments include allergen avoidance, pharmacological treatments and allergen-specific immunotherapies (Alvaro et al, 2013). Pharmacological treatments do not cure the disease, but control the symptoms and deterioration of the disease. Allergen-specific immunotherapies are currently accepted as only curative treatment approaches for patients with atopic asthma. In addition, symptoms can be improved by controlling the environmental factors, partnering with patients and families, and assessing the severity of the disease. According to the severity of asthma, using correct type and dose of medication is important. There are two types of medications used for asthma treatment; one for acute symptoms and the other for long term actions of the disease (Spickett, 2013).

The asthma severity defines the intensity within the process of the disease. The asthma severity assessment is used as a guidance for the decision of the medication and its correct therapeutic dosage. Asthma medications can be administered by either local (inhaled) or systemic (ingested or parenteral) routes. The major advantage of inhaled drugs is to get into the airways directly and can be given at higher concentrations, as it has minimal systemic side effects. The main treatments are inhaled short / long-acting bronchodilators (β-2 andrenergic agonists) and inhaled corticosteroids (Adelman *et al*, 2012).

Inhaled short acting β-2 andrenergic agonists (SABAs) are used for the fast relief of acute asthma symptoms. The effects of β-2 andrenergic agonists include bronchodilation, inhibition of mast cell mediator release and muco-ciliary clearance. However, inhaled long-acting β-2 andrenergic agonists (LABAs) provide longer duration (up to 12hr) of bronchodilation than SABAs. LABAs should not be used in the acute phase of asthma or exacerbation and should not be used as a monotherapy for asthma treatment in the long run. They are best used together with inhaled corticosteroids (ICS) for further control of symptoms in patients with moderate to severe asthma (Adelman *et al*, 2012).

Corticosteroids are the most important class of medication used for the control of asthma symptoms as they have broad anti-inflammatory effects (Adelman et al, 2012). Corticosteroids exert their effects by various ways including modulation of nuclear regulatory proteins, catecholamine receptors and transcription of pro-inflammatory cytokines i.e. IL4, IL6, IL3, IL8. They inhibit the synthesis of inflammatory lipid mediators; leukotrienes (LTs) and prostaglandins by increasing the lipocortin-1. They also stop the mucosal secretion in airways.

ICSs are the first-line agent for all forms of asthma. The achievement of treatment can be gained with low dose of ICSs. However, patients with severe asthma gain benefits from high dose therapy. Combination of ICS with other therapies are being used for many asthma patients for example ICS+LABA and ICS+ leukotriene receptor antagonists (LTRA). LTRAs are the antagonists for $CysLT_1$ receptor at where LTs exert their effects in asthmatic cases. They are administered orally.

Other treatments include omalizumab which is a humanized monoclonal antibody that binds to $F_C\varepsilon RI$ receptor on IgE and forms an immune complex. Therefore, IgE is no longer able to bind to mast cells and basophils. This inhibits those cells from activation by an allergen. Omalizumab is currently approved for usage of patients above the age of 12 with moderate to severe asthma. Omalizumab is administered by injection into the subcutaneous region at every 2 to 4 weeks depending on the patient's total IgE titre and weight. Treatment is administered to patients with total IgE levels above 30 IU/ml and who have IgE mediated hypersensitivity to house dust mites, cockroaches, animal dander or mould.

Allergen-specific immunotherapy is the only curative treatment option that have been used for allergic asthma. Studies show that there is an improvement in asthma symptoms both in children and adults. Allergen specific immune therapy involves the administration of increasing doses of allergen in order to manipulate the immune system and induce long term tolerance. The objective of immunotherapy is to skew the response from Th2 cells to Th1 cells which secrete IL10 and TGF-β for reducing the production of IgE antibodies. (Adelman *et al*, 2012)

Allergen immunotherapy can be administered under the tongue (with drops or tablets) which is called sublingual immunotherapy (SLIT) or by subcutaneous injections which is called subcutaneous immunotherapy (SCIT). SLIT is used as

a safer alternative to SCIT for treatment of allergic asthma as SCIT has potential adverse systemic side effects. (Cave and Atkinson, 2014)

### 2.8. Stepwise Management of Childhood Asthma

Asthma is the most common chronic disease among children worldwide and is characterized by episodes of cough, wheezing, and shortness of breath. Asthmatic children generally experience daytime fatigue and reduced activity levels delaying their developmental progress and reducing the socialization of the children (Alvaro et al., 2013).

Diagnosis of asthma in young children is difficult because respiratory symptoms such as wheezing and cough are not asthma specific and can be seen in children suffering from other diseases. There are no tests that can certainly diagnose asthma in young children. Therefore, the diagnosis of childhood asthma is based on symptoms' patterns (wheezing, cough, shortness of breath, activity limitations and nocturnal symptoms) and their frequencies together with accurate family history and physical findings of the patient.

The goal of asthma treatment in children is to manage the optimization of the lung function, reducing the day and night-time symptoms and reducing the limitations in day-time activities. It should also decrease the need for using the reliever treatment by reducing exacerbations. In children, it is important to achieve the management with minimum medication side effects (van Aalderen, 2012).

Management of asthma requires four key components to assess the disease and control its symptoms. These components include, monitoring of severity, patient education, controlling external inducers and medications.

The Global Initiative for Asthma (GINA) was established in 1993 in the collaboration with National Heart, Lung and Blood Institute (NHLBI) and World Health Organization. The major aims of the GINA include publishing the information about asthma management and giving the clinically useful strategies in the guidance of scientific evidence. GINA has been regularly published and global strategies for asthma management and prevention based on many national guidelines have been annually updated (Reddel et al., 2015).

## 2.9. Control-Based Asthma Management

The asthma treatment is accustomed in a continuous cycle; assess, adjust treatment and review response (Figure 2) (Reddel et al, 2015)

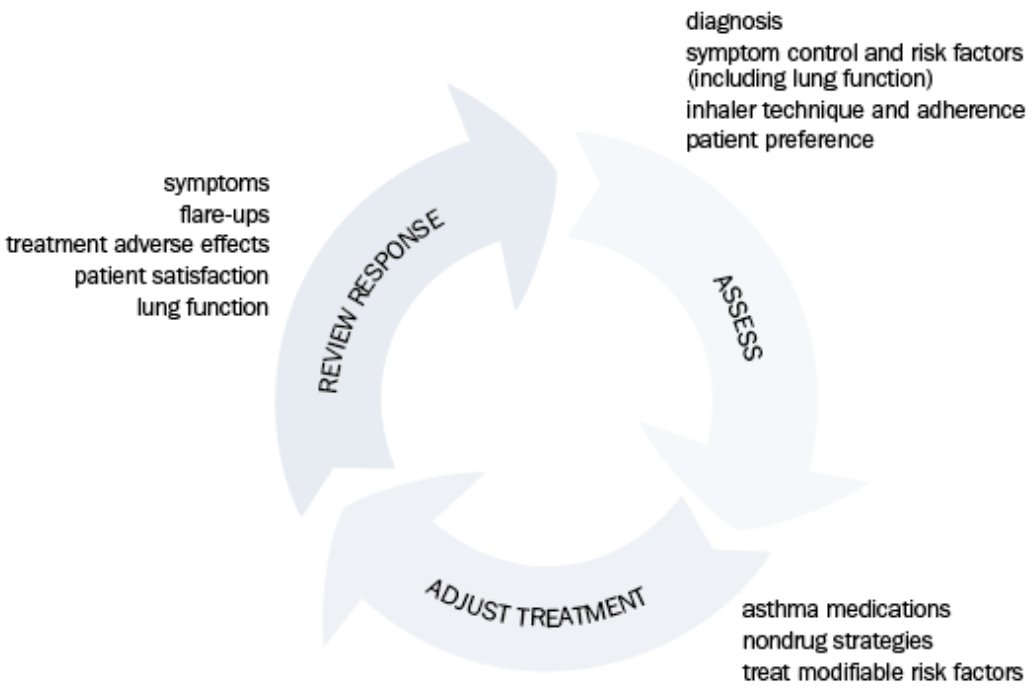


**Figure 2:** The GINA Cycle of Asthma Management (GINA guidelines, 2015)

### 2.10. Assessment of Asthma

Asthma control means the extent to which manifestations of asthma are reduced or removed, by treatment and close follow-up. There are two components of asthma control; symptom control (the child's asthma status over the previous 4 weeks) and future risk (how asthma may affect the child in the future). Both of these components should be monitored in order to complete the picture of the child's asthma condition. The assessment of children could be problematic as the assessment is dependent on the reports of the family members and care givers (GINA guidelines, 2015)

### 2.11. Stepwise Treatment of Asthma

The asthma treatment is a stepwise approach in which the medication dosages are adjusted according to the patient's asthma status to achieve good symptom control and reduce the future risk of exacerbations and medication side effects as shown in Figure 3. (GINA guidelines, 2015)

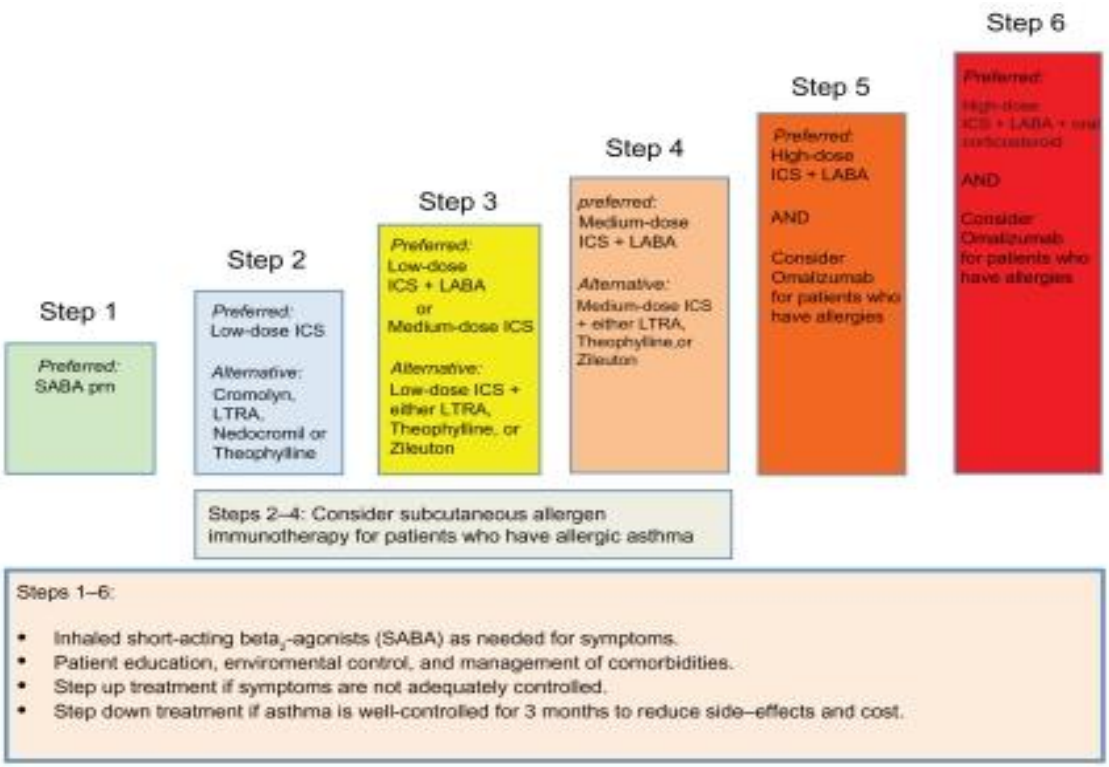


**Figure 3:** Stepwise treatment of asthma. Step 1: Wheezing children should use inhaled SABA. Step 2: Low dose of ICS should be given at least 3 months to establish its effectiveness. Step 3: Other treatment option is to use LABA with the low dose of ICS or use only medium dose of ICS. Step 4: LTRA may be considered as an additive medication to the medium dose ICS. Step 5/6: Doubling the initial dose of ICS and consider alternative technique. (GINA guidelines, 2015).

If the patient's response to 3 months of symptom control therapy is not enough to control the disease progress and/or persisting exacerbations, before stepping up the medication, other possible reasons should be eliminated. For example;

- ✓ exposure to the tobacco smoke or allergen
- ✓ check about the correct inhaler technique
- ✓ confirm whether the patient is taking the correct dose and frequency of medications
- ✓ confirm that the symptoms are due to asthma, and not because of an alternative condition.

### 2.12. Reviewing of the Treatment Response

Clinicians should assess the asthma symptom control, risk factors and side effects of medication at every visit. (GINA guidelines, 2015)

# 3. MATERIAL AND METHODS

## 3.1. Study Population

A retrospective study was conducted from January 2012 and December 2014 in Division of Paediatric Allergy and Immunology in the Near East University Hospital, Cyprus, which is the only tertiary centre for paediatric allergy and immunology in North Cyprus.

The medical records of patients with recurrent wheezing who had undergone a quantitative serum Ig analysis during follow-up period were reviewed. Inclusion and exclusion criteria were as follows.

The inclusion criteria were: children (i) with recurrent wheezing, cough and/or croup who were unable to decrease inhaled steroid dose for control of symptoms (partially controlled) according to guideline recommended treatment (GINA guidelines,2015), (ii) who were investigated for immunodeficiency (iii) who had a follow up period more than 6 months (iv) who did not receive allergen-specific immunotherapy.

The exclusion criteria were: Children (i) with only recurrent infections (Paul &Shearer,1999; Woroniecka & Ballow,2000; Buckley,2011) (ii) who had co-morbidities (such as gastroesophageal reflux, sinusitis or cystic fibrosis).

The demographic, clinical and laboratory findings of the children who were included in the study had been collected from the patient files retrospectively.

Information including gender, age, family history of İmmunodeficiency and allergy, treatment, follow up period, remission of hypogammaglobulinemia*, initial and final treatments, serum Ig levels, IgG subgroup levels, lymphocyte subset percentages, specific antibody responses, skin prick test or allergen specific IgE results had been recorded and evaluated statistically.

Asthmatic children were divided into two groups based on the concentration of their serum Ig levels. HGG was defined as the serum Ig values lower than 2SD of mean for age and gender-matched controls (Aksu et al, 2006)

*****Patients whose Ig levels are normalised within 2 years.

### 3.2. Study Design

The medical records of 286 children whose serum Ig levels had been analysed previously were overviewed. Among those 286 children, 125 of them met the inclusion and enrolled into the study. The remaining 161 children were excluded from the study. One hundred and forty had only recurrent infections without any asthmatic symptoms, while 3 were excluded due to loss of follow-up, and 18 had co-morbid diseases (except for HGG) complicating asthma course. The study design is shown below in Figure 4. Further clinical evaluation prompted the analysis of serum IgG subclasses of 63 patients and lymphocyte subset analysis of 25 patients.

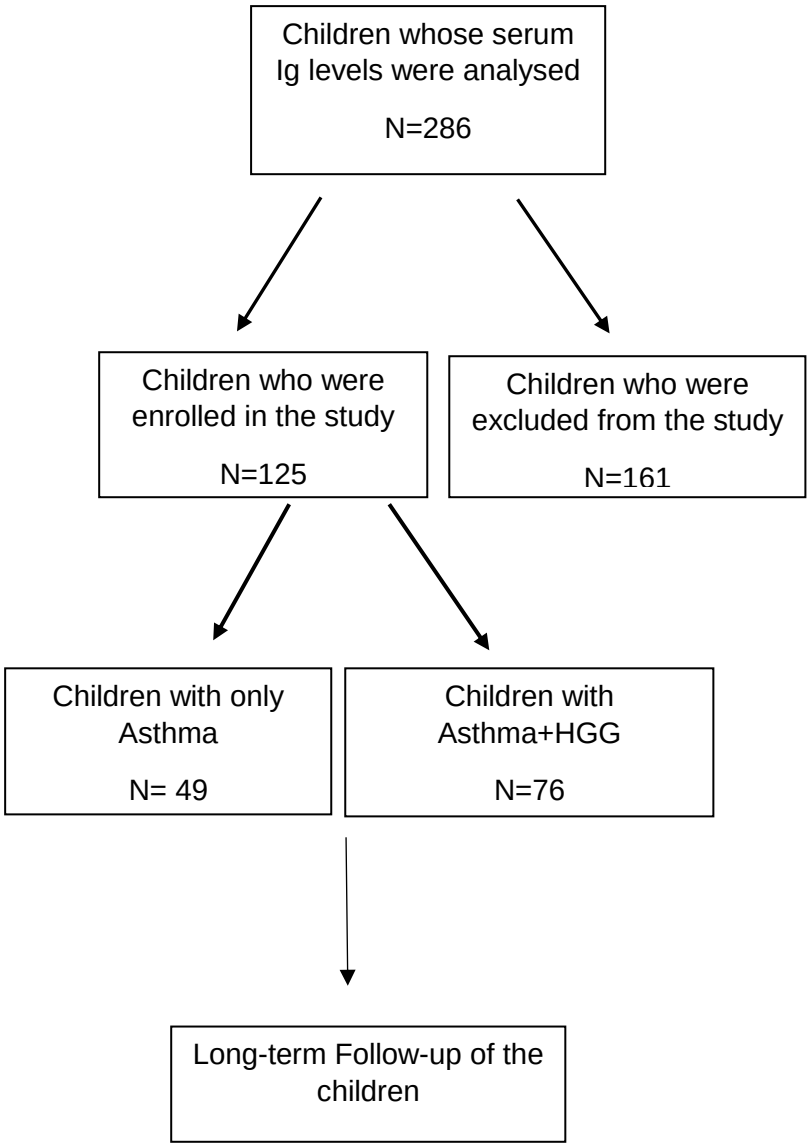


**Figure 4:** Scheme of the study design. Among 286 patients whose Ig titres were analysed, 125 of them were included in the study and 161 were excluded. 49 out of 125 were asthmatic children and 76 of 125 were asthmatic children with hypogammaglobulenemia.

### 3.3. Follow-up Period and Treatment

According to the follow-up protocol established by the head of Division of Paediatric Allergy and Immunology of Near East University Hospital; asthmatic children with recurrent wheezing, cough and croup who partially respond or do not respond to the standard asthma medications and environmental precautions, should be further evaluated in order to rule out co-morbidities including gastroesophageal reflux, sinusitis, cystic fibrosis, and immune deficiency diseases before accepting the patient as a severe asthma case. Gastroesophageal reflux was ruled out based on history and unresponsiveness to anti-reflux treatment. Sinusitis was ruled out based on history revealing prolonged purulent nasal discharge, postnasal dripping, wet cough and physical examinations. For cystic fibrosis, sweat test was performed.

Then evaluation of the immune system is performed including the measurements of the serum Ig and IgG subclass levels, was performed further tests such as determination of the lymphocyte subset percentages and specific antibody responses were performed if indicated by the clinician.

For the treatment of asthma, ICS was used as a first line medication. The dosage of ICS was adjusted according to the severity of symptoms based on the guideline recommendations Patients were controlled every 2 months in order to adjust the ICS dosage. All of the clinical follow-up and work-up throughout the study was performed by the same investigator.

## 3.4. Immunologic and Other Laboratory studies

### 3.4.1. Atopy

#### *3.4.1.1. Skin Prick Test*

Skin prick tests (SPT) were performed with 21 common aero-allergens including *Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alternaria, Aspergillus mix, Penicillium mix, Candida albicans, Betulaceeae, Aesculus hippo, Olea europea, Plantago, Artemisia, Parietaria, Secale cereale, Triticum vulgaris, Acacia dealbata,* and mixture of five grasses, feathers, cat hair, dog hair and cockroach (Stallergenes, Antony, France). Histamine and saline were used as positive and negative controls, respectively. A drop of each allergen extract was placed on the volar surface of the left forearm and penetrated with a staller point. After 15 minutes, the wheal reaction was measured as the mean of the longest diameter and the diameter perpendicular to it. A wheal diameter of at least 3 mm greater than those of the negative control was considered positive.

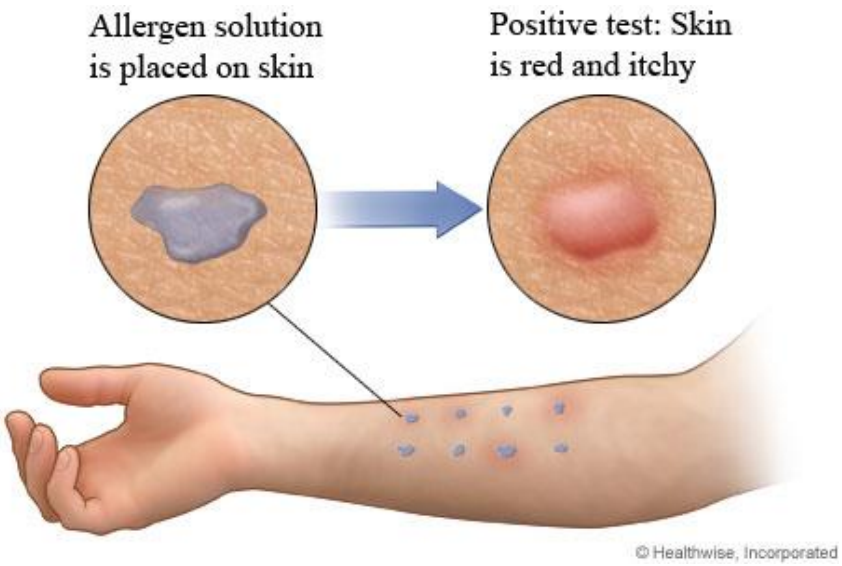


**Figure 5:** Skin prick test. A drop of each allergen extract was placed on the volar surface of the left forearm and penetrated with a staller point. After 15 minutes, wheal and flare reaction observed. (myhealth.alberta.ca)

#### 3.4.1.2. Allergen-specific IgE Antibody Test

The patient's blood sample (5ml) were taken into blood collection tubes and centrifuged at 1500 g for 10 minutes to obtain serum part of the blood.

Specific IgE antibodies of suspected or known allergens for the purpose of guiding a diagnosis about allergy were detected from patient's serum by using the radioallegosorbent test (RAST).

RAST is scored on a scale 0 to 6. Scale 0 means the absent or undetectable level of specific IgE and scale 6 defines extremely high levels of specific IgE.

**Table 1:** RAST Rating Scale.

| RAST rating | IgE level (kU/L) | Comment |
|---|---|---|
| 0 | < 0.35 | Absent or undetectable allergen specific IgE |
| 1 | 0.35 – 0.69 | Low level of allergen specific IgE |
| 2 | 0.70 – 3.49 | Moderate level of allergen specific IgE |
| 3 | 3.50 – 17.49 | High level of allergen specific IgE |
| 4 | 17.50 – 49.99 | Very high level of allergen specific IgE |
| 5 | 50.00 – 100.00 | Ultra high level of allergen specific IgE |
| 6 | > 100.00 | Extremely high level of allergen specific IgE |

### 3.4.2. Assessment of immunoglobulin Levels

For all patients, the initial diagnostic work up started with complete blood count in order to rule out neutropenia and lymphopenia. Complete Blood counts were performed with Sysmex XT- 2000*i* Automated Haematology Analyser (Sysmex, Mississauga, Ontario, Canada.)

For measuring the serum Ig levels, 10ml of patient's blood samples were centrifuged at 3200g for 10 minutes. After the separation of the serum part, serum IgG, IgA, IgM levels were measured by turbidimetric method using Roche Cobas c311 and commercially available kits. (Roche Diagnostics, Mannheim, Germany).

Serum IgE levels were measured by fully automated ELİSA Roche cobas 411 using commercially available kits (Roche Diagnostics, Mannheim, Germany).

The quantification of serum immunoglobulin subclasses (IgG1, IgG2 and IgG3) were performed by nephelometry with commercially available lits. Results were evaluated according to the normal ± 2SD values based on age for Turkish children (Aksu et al, 2006). Values lower than 2SD were accepted as low. All measurements were performed by the same investigator.

### 3.4.3. Lymphocyte Subset Analysis

Lymphocyte subpopulation; total lymphocytes (CD45), total T-cells (CD3), helper T-cells (CD3+/CD4+), cytotoxic T-cells (CD3+/CD8+), B-cells (CD19+), NK cells (CD16+/56+), and Active T-cells (CD3+/HLA DR+) was analysed via flow cytometry (BD FACS Calibur, BD Biosciences, San Jose, CA, USA).

For each patient; blood samples obtained in heparinized tubes are used in 6 different tubes labelled according to markers used. 1$^{st}$ tube was isotype control. 10µl of surface marker and 100µl of peripheral blood were mixed and incubated for 20 minutes at room temperature (RT). At the end of incubation 2ml of lysing solution was added to each tube for the elimination of red blood cells and tubes were incubated for 10 minutes at RT. After incubation, tubes were centrifuged for 7 minutes at 1500g. The supernatants were discarded and 2ml of cell wash solution was added to each tube. Tubes were then centrifuged for 7 minutes at

1500g. Supernatants were then discarded and 500µl of cell wash solution was added to all tubes for analysis.

During analysis, lymphocytes were gated according to CD45 surface marker. Then, percentages of all markers were detected. Results were evaluated according to the normal ± 2SD values based on age for Turkish children (Ikinciogullari et al, 2004). Values lower than 2SD were accepted as low.

Measuring antibody production in response to antigen exposure or vaccination is key to disease prevention and treatment. Antigen specific antibody responses were evaluated by anti-tetanus toxoid IgG antibody level after 2 or 3 weeks following the vaccination, EuroImmune IgG ELISA, Pneumococcus IgG, ELİZEN/ ZenTech (catalogue no:E-DG-96) and anti- Hbs ,fully automated ELİSA Roche Cobas 411 (catalogue no: El 2060-9601 G), with commercially available kits (Roche Diagnostics, Mannheim, Germany.)

Note that, all the equipment used for the analyses of Ig levels, lymphocyte subsets, antigen specific antibody responses is calibrated routinely in the Laboratory of the Near East University Hospital. In addition, control samples are routinely used prior to analysis of the patient serum samples.

### 3.4.4. Antigen-specific Antibody Response

Antigen specific antibody responses were evaluated by anti-tetanus toxoid IgG antibody level, EuroImmune IgG ELISA, Pneumococcus IgG, ELİZEN/ ZenTech (catalogue no:E-DG-96) and anti- Hbs ,fully automated ELİSA Roche Cobas 411 (catalogue no: El 2060-9601 G), with commercially available kits (Roche Diagnostics, Mannheim, Germany).

### 3.4.5. Statistical Analysis

Statistical analyses were performed using the IBM SPSS software package for Windows (release 20.0.0, SPSS Inc., Chicago, Ill, USA). Descriptive statistics were expressed as mean (SD), median and range. Prevalence rates were expressed as percentages. Categorical data were analysed using the chi-square, Kruskal-Wallis and Mann Whitney U tests. P values less than 0.05 were considered statistically significant.

# 4. RESULTS

## 4.1 Demographic Characteristics of Patients

The age range and male percentage of the125 patients who were included in our study was 5-137 months and 63.2%, respectively. Family history of allergic diseases, primary immune deficiency diseases and consanguineous marriage of parents were found to be 54.9%, 4.9% and 2.5%, respectively. Although, the reason of admission of those 125 children to the Paediatric Allergy and Immunology Division were allergic complaints including recurrent wheezing 13 (10.4%), recurrent cough/croup 13 (10.4%), both of them 89 (71.2%), any of those + recurrent infections 10 (8%), 76 (60.8 %) of them were diagnosed as having HGG. In other words, the majority of patients (92%) admitted with asthma–like symptoms, while only 8% of them had a history of a recurrent infection in addition to asthma-like symptoms. Demographic characteristics of those 125 children who were included, is presented in Table 2.

**Table 2:** Demographic characteristics of the study population.

| **Characteristics** | **N (%)** |
|---|---|
| **Age (months) (min-max (mean±SD)** | (5-137) (41,3±25.2) |
| **Gender (Male/Female)** | 79/46 (63.2/36.8) |
| **Family History**<br>**-Allergy**<br>**-İmmune deficiency**<br>**-Consanguineous marriage** | <br>67 (54.9)<br>6 (4.9)<br>3 (2.5) |
| **Diagnosis of PID*** | 76 (60.8) |
| **Reason of admission**<br>**-recurrent wheezing**<br>**-recurrent cough/croup**<br>**- Both**<br>**-Any of above + recurrent Infection.** | <br>13(10.4)<br>13(10.4)<br>89(71.2)<br>10(8.0) |
| **Follow-up period (min-max) (mean±SD)** | (2-48) (12.1-12.28) |
| **Atopy*** | 55(67.1) |

*PID: Primary immune deficiency

*Atopy: Defined as skin prick test positivity or high allergen-specific IgE.

### 4.2. Comparison of patients with and without HGG

When children with and without HGG were compared based on age, gender, admission complaints, family history of allergy, immune deficiency and consanguineous marriage, no statistically significant differences were detected. On the other hand, serum total IgM, IgG and IgG3 were statistically significantly lower in the HGG group (p=0.016, p=0.000, p=0.000, respectively). Although there was no significant difference between the serum total IgE levels between the asthmatic children with and without HGG (p=0.638), the asthmatic children with PID were significantly more atopic (P=0.044). Characteristics of asthmatic children with and without PID are presented in Table 3, and Figures 6 (a-e).

**Table 3.** Characteristics of children with and without HGG.

| | **Patients with PID N (%)** | **Patients without PID N (%)** | **P value** |
|---|---|---|---|
| **Gender (Male)** | 50/26 (65.8/34.2) | 29/20 (59.2/40.8) | 0.569 |
| **Reason of admission**<br>**-recurrent wheezing**<br>**-recurrent cough/croup**<br>**- Both**<br>**-Any of above + recurrent Infection.** | <br>7 (9.2)<br>7 (9.2)<br>56(73.7)<br>6 (7.9) | <br>6(12.2)<br>6(12.2)<br>33(67.3)<br>4(8.2) | 0,874 |
| **Family History**<br>**-Allergy**<br>**-Immune deficiency**<br>**-Consanguineous marriage** | <br>39(53.4)<br>6(8.2)<br>3(41.1) | <br>28(57.1)<br>0(0)<br>0(0) | <br>0.686<br>0.040*<br>0.151 |
| **Low IgA** | 14 (18.4) | 1(2) | 0.065 |
| **Low IgM** | 21(27.6) | 0(0) | 0.016* |
| **Low IgG** | 39(51.3) | 1(2) | 0.000* |
| **Low IgG2** | 1(2.3) | 1(5.3) | 0.087 |
| **Low IgG3** | 32(74.4) | 0(0) | 0.000* |
| **High total IgE** | 30(50.3) | 20(51.3) | 0.638 |
| **Atopy** | 39 (75.0) | 16(53.3) | 0.044* |

* Statistically significant, $p<0.05$

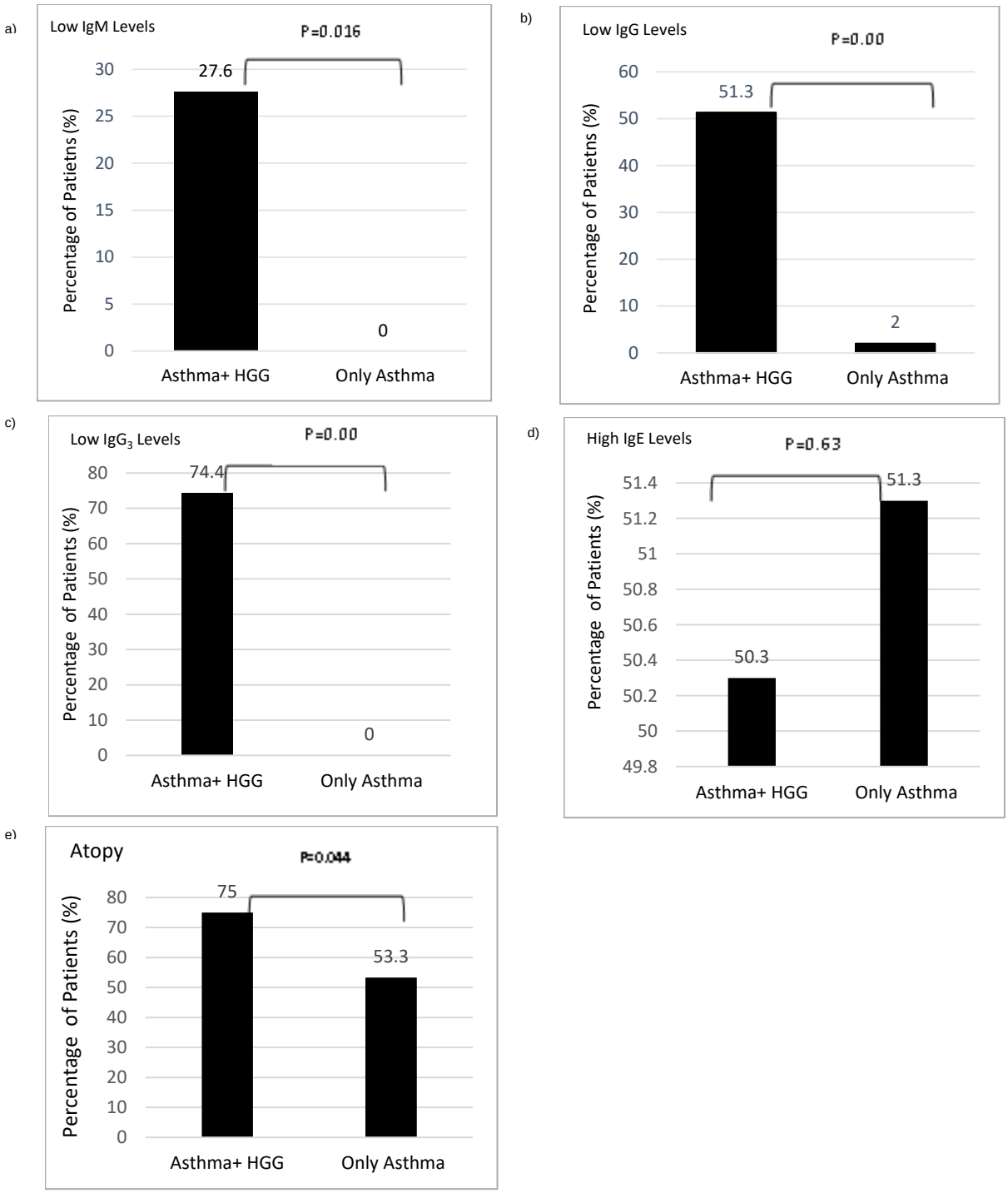


**Figure 6:** Comparison of Ig levels and presence of atopy in both groups; a-c) Percentages of low IgM, IgG and $IgG_3$ were more frequent in the Asthma+ Hypogammaglobulinemia (HGG) group d) Serum IgE levels of the asthmatic patients with and without HGG were similar. e) Comparison of atopic status of asthmatic children with and without HGG revealed significantly more atopic sensitization in those who had asthma+ HGG.

### 4.3. Characteristics of Patients with PID Diseases

The most frequent diagnosed PID diseases among the asthmatic children with hypogammaglobulinemia were transient hypogammaglobulinemia (46%), followed by IgG subclass deficiency (32.9%). Distribution of immune deficiency diseases is presented in table 3.

**Table 4:** Distribution of diagnosed Immune Deficiency Diseases

| Immune Deficiencies | N (%) |
|---|---|
| Transient Hypogammaglobulinemia of Infancy | 35(46.1) |
| IgG subclass deficiency | 25(32.9) |
| IgA deficiency | 1(1.3) |
| Common Variable Immune Deficiency | 7(9.2) |
| IgM deficiency | 6(7.9) |
| Protracted Hypogammaglobulinemia of Infancy | 1(1.3) |
| Others | 1(1.3) |

**Table 5:** Immunological findings in children diagnosed as PID disease.

| | **Transient HGG of Infancy (n=35)** | **IgG subclass deficiency (n=25)** | **IgA deficiency (n=1)** | **CVID (n=7)** | **IgM deficiency (n=6)** | **Protracted HGG of Infancy (n=1)** | **Others (n=1)** | **Total No of patient (n=76)** |
|---|---|---|---|---|---|---|---|---|
| **Low IgG** | 33 (84.6%) | 1 (2.6%) | 0 (0%) | 4 (10.3%) | 0 (0%) | 1(2.6%) | 0(0%) | 39 |
| **Low IgA** | 8 (57.1%) | 0(0%) | 1 (7.1%) | 5 (35.7%) | 0(0%) | 1(4.8) | 0(0) | 14 |
| **Low IgM** | 7(33.3) | 2(9.2) | 0(0) | 5(23.8) | 6(28.6) | 1(4.8) | 0(0) | 21 |
| **Low IgG1** | 3(42.9) | 2(28.6) | 0(0) | 2(28.6) | 0(0) | 0(0) | 0(0) | 7 |
| **Low IgG2** | 1(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1 |
| **Low IgG3** | 7(21.9) | 22(68.8) | 0(0) | 1(3.1) | 1(3.1) | 1(3.1) | 0(0) | 32 |
| **High total IgE** | 10(33) | 14(46.7) | 0(0) | 3(10) | 3(10) | 0(0) | 0(0) | 30 |
| **Low CD45** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 22 |
| **Low CD19** | 0(0) | 0(0) | 0(0) | 1(50) | 0(0) | 0(0) | 1(50) | 2 |
| **Low CD3** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1(100) | 0(0) | 1 |
| **Low CD4** | 0(0) | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 0(0) | 1 |
| **Low CD8** | 0(0) | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 0(0) | 1 |
| **Low CD16/56** | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 2 |
| **Low Active T** | 4(80) | 0(0) | 0(0) | 0(0) | 0(0) | 1(20) | 0(0) | 5 |

Among the 125 children evaluated, 76 of them had comorbid HGG. Of those 76 children, 39 children had been demonstrated to have low IgG, 14 had low IgA, 21 had low IgM, 32 had low IgG3 levels. Distribution of immunological results based on clinical diagnosis is presented in table 4.

High IgE level was detected in 32 among 76 PID children, the majority being diagnosed as IgG subclass deficiency (Table 4)

Lymphocyte subset analysis results were normal for all PID patients except for 3 patients diagnosed as CVID; 1 with low CD4, 1 with low CD19, 1 with low CD8 levels. (Table 5)

Specific antibody responses including Tetanus (1 patient), pneumococcus (1 patient) and hepatitis B (2 patients) were low in CIVD patients. In addition, isohaemagglutinin antibody response was detected low in 3 of CVID patients (data not shown).

### 4.4. Treatment

Asthmatic children with and without PID HGG were compared based on their “asthma” and “HGG” treatments.

All of the participants had asthmatic symptoms, therefore, they were being treated with as needed beta-2 agonists, inhaled corticosteroids or montelukast sodium as prophylaxis (Table 5). No statistically significant differences between the groups at the enrolment based on the asthma medications needed for control of asthma symptoms. Asthmatic children with co-morbid HGG received additional treatments such as prophylactic antibiotics, IVIG plus prophylactic antibiotics.

Decision of treatment was based on the patients’ clinical conditions. In case of, severe and recurrent infections or secondary complications due to infections, IVIG was initiated. For moderate recurrent infections, prophylactic antibiotic treatment was prescribed. Those with mild recurrent infections such as upper respiratory tract infections were followed up without any medication for hypogammaglobulinemia (Table 6).

**Table 6:** Distribution of treatments that were given to patients in both groups.

| | **Asthma + HGG N (%)** | **Only Asthma N (%)** | **P value** |
|---|---|---|---|
| **Asthma treatment**<br>- ICS<br>- Montelukast-Na | 46(62.9)<br>5(6.8) | 23(52.5)<br>8(18.2) | 0.767<br>0.107 |
| **PID Treatment**<br>-Trim-Sulf<br>- IVIG + Trim-sulf | 28(38.3)<br>2(2.7) | 0(0)<br>0(0) | ---- |

ICS: Inhaled Corticosteroid

Trim-Sulf: sulfamethoxazole and trimethoprim

IVIG: Intravenous Immunoglobulin

HGG: Hypogammaglobulinemia

### 4.4.1. Use of ICSs

Two groups of patients were compared based on the mean daily dose of ICSs needed to control symptoms at enrolment, at the end of study and within both groups. The results revealed that, although the dose of ICSs decreased in both groups, the dose of ICS was statistically significantly decreased in the asthma+ hypogammaglobulinemia group (P= 0.024), whereas there was no significant difference in the only asthma group (P=0.068) (Figure 7).

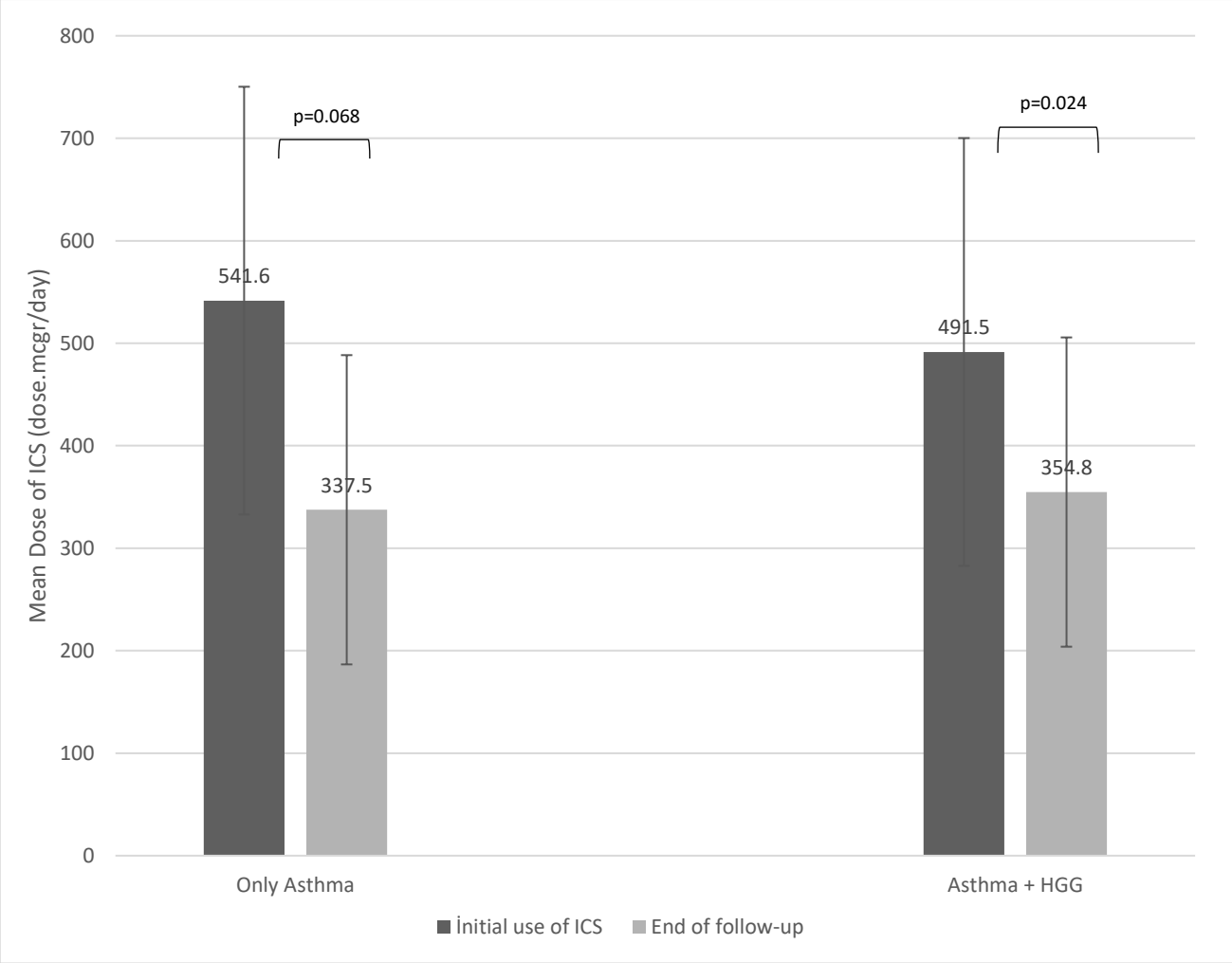


**Figure 7**: Comparison of mean daily dose of ICS within groups. The mean dose of ICSs decreased in both groups however, according to p values, it was statistically significant in asthma+ HGG group.

### 4.4.2. Use of Trim-Sulf or IVIG

Within the asthma+ HGG group of 70 patients, 28 of them (38.3%) were under prophylaxis of Trim-Sulf. Two of them (2.7%) were given both IVIG and Trim-Sulf.

# 5. DISCUSSION

In the current study, we demonstrated that among 125 asthmatic children under treatment with ICSs and /or montelukast-sodium with partial response or non-responsive to medications, 76 of them (60.8%) were demonstrated to have a co-morbid hypogammaglobulinemia disease.

Interestingly, atopy was higher in the asthma + hypogammaglobulinemia group than the only asthma group and as expected, antibody levels were lower in those with hypogammaglobulinemia.

The most frequent co-morbid PID diseases were found to be transient hypogammaglobulinemia of infancy and IgG subclass deficiency.

The daily dose of inhaled corticosteroids for asthma control in the children with asthma + comorbid HGG decreased significantly after the initiation of appropriate treatment for HGG. Although, comparison of percentage use of ICSs was not significantly different between the two groups at the initial evaluation, the dose of ICS was significantly decreased in the asthma+ HGG group, whereas there was no significant difference in the only asthma group at the end of the follow-up period. It was thought that additional immune prophylaxis treatment probably prevented recurrent infections that triggered the asthma symptoms in children with co-morbid hypogammaglobulinemia. This probably helped decreasing the percentage use of ICSs throughout the treatment period.

Little is known about the characteristics of asthma children with HGG. In a study that was performed in adults, prevalence of HGG in adult asthmatic patients was reported as 25%, while in our study the prevalence of HGG in paediatric asthma patients was detected as 60.8%, 35 of them were diagnosed as THGI (28%). After the exclusion of the THGI patients the prevalence was re-calculated as 32%, which is in accordance with the results of the adult study (Dupın et al,2016) It is difficult to compare our study to previous investigations due to heterogeneity of age groups and severity of asthmatic children in the other studies published to date. In the study of Öner *et al*., all children with recurrent wheezing regardless of severity and/or response to treatment who were between 9-24 months and 2-6 years of age groups were included and compared with healthy controls based on Ig and Ig subgroup levels. They demonstrated that recurrent wheezy children

who were belonged to the 2-6 years age group had significantly lower IgG3 levels compared to their healthy counterparts (Oner et al,2000)

Another study included all children with asthma under follow-up, regardless of severity and studied immunoglobulin levels. Then, they compared the features of asthmatic children with normogammaglobulinemia and hypogammaglobulinemia. Their results revealed that children with hypogammaglobulinemia had earlier onset of disease, lower rates of atopy and earlier clinical improvement compared to the group having normogammaglobulinemia (Baris *et al, 2011)*. Another study reveal that, the long term follow up of children with low $IgG_3$ levels could be susceptible to asthma or other allergic diseases (Ones et al, 1998). Although those studies reported abnormalities in IgG and IgG subclass levels was related with wheezing, they did not differentiate between clinical phenotypes and severity of asthmatic children. The importance and strategies that should be taken in the treatment of children with different asthma severity cannot be deduced. On the other hand in our study, children of all age groups having mild to moderate persistent asthma, who were partly or unresponsive to standard asthma treatment were included. Our results suggested that, before increasing dose of ICS or other asthma treatments those non-responder children should be evaluated for co-morbid HGG. In addition results of all those three studies clearly demonstrated that HGG in children may present with isolated asthma symptoms. The first study reported a 66.6% of $IgG_3$ deficiency among recurrent wheezers, while the study of Baris *et al* has reported 36% hypogammaglobulinemia among children with asthma. In accordance with those results 60.8% of our group had co-morbid HGG. The current study was performed in paediatric Allergy-Immunology Division of a University Hospital. This may be the reason for the high HGG rates in asthmatic children. Based on those high hypogammaglobulinemia rates, children who have deficient response to the guideline based asthma treatment plans during long-term paediatric asthma follow-up, underlying immune deficiency should be considered. There are limited published data in the literature on this subject.

In our study, serum total IgM, IgG and $IgG_3$ levels were statistically significantly lower in the PID+ asthma group (p=0.016, p=0.000, p=0.000, respectively) as expected. Atopy and high IgE levels were distributed equally in both groups of children, in contrast to the study performed previously (Barıs et al, 2011) showing

that low immunoglobulin levels can be detected in a significant proportion of children with asthma, especially in non-atopic cases. They demonstrated that lower rates of atopy was present in asthmatic children with PID disease (17.9%) with earlier clinical improvement accompanied with discontinuation of ICS when compared to children with normal Ig levels. However, we have shown that there is no strict association between non-atopy and low immunoglobulin level in asthmatic children.

Also, Barış *et al*, the patients were classified depending on the presence of HGG (Barıs et al, 2011) .However, no further immunological evaluation were not reported for comparison based on type of immune deficiency. In our study, it was demonstrated that the most frequent diagnosed PID diseases among the asthmatic children with hypogammaglobulinemia were transient hypogammaglobulinemia (46%), followed by IgG subclass deficiency (32.9%).

In accordance with the results of Barıs et al, addition of appropriate PID treatment in children with asthma+ PID, resulted in significant decrease of the daily consumption of inhaled corticosteroids (Barıs et al, 2011).In accordance, diagnosis of PID in asthmatic children unresponsive to ICS treatment allowed an earlier response and reduction in dose of ICS in our study. Currently there is limited published data on HGG as an underlying cause of uncontrolled childhood asthma. Our study underlines the importance of the immunological evaluation in asthmatic children unresponsive to standard treatment.

It is well known that symptoms of children with only asthma improve by age, especially in those with non-atopic (Sly et al, 2008; Barıs et al, 2011)

One of the limitations of the study was that, it was not possible to include a control group of children with asthma + PID due to ethical issues. Diagnosis of any PID disease prompts appropriate treatment, in order to prevent long term irreversible changes in airways such as bronchiectasis (Spickett, 2013; Moise et al,2010)Also another limitation was that, an additional control group of asthmatic children who are responsive to the standard treatment was not included and could not be compared as generally further immunological tests are not a necessity for responsive children and is not a routine investigation in our department.

## 6. Conclusion

In conclusion, the current study demonstrated that, majority of asthmatic children who continue to have symptoms despite of appropriate guideline based treatment, may have co-morbid immunological abnormality. Treatment of co-morbid hypogammaglobulinemia improves asthma control which enables the reduction of ICS doses in those children. These results underline the necessity of immunological evaluation in uncontrolled childhood asthma in order to i) prevent the long term side effects of high dose ICSs, ii) prevent the frequency and severity of asthma symptoms, and iii) decrease the morbidity and improve the quality of life.

## 7. References

Adelman, D. C., Casale, T. B and Corren, J. (2012). Manual of Allergy and Immunology. 5th ed. Philadelphia: Lippincott Williams & Wilkins.

Agondi, R. C., Barros, M. T., Rizzo, L. V., Kalil, J. & Giavina-Bianchi, P. 2010. Allergic Asthma in Patients with Common Variable Immunodeficiency. *Allergy,* 65**,** 510-5.

Aksu, G., Genel, F., Koturoglu, G., Kurugol, Z. & Kutukculer, N. 2006. Serum Immunoglobulin (Igg, Igm, Iga) and Igg Subclass Concentrations in Healthy Children: A Study Using Nephelometric Technique. *Turk J Pediatr,* 48**,** 19-24.

Alvaro, M., Sancha, J., Larramona, H., Lucas, J. M., Mesa, M., Tabar, A. I., Martinez Canavate, A., Immunotherapy Working, G. & Sociedad Espanola De Inmunologia Clinica Y Alergia, P. 2013. Allergen-Specific Immunotherapy: Update on Immunological Mechanisms. *Allergol Immunopathol (Madr),* 41**,** 265 72.

Ballow, M. 2002. Primary Immunodeficiency Disorders: Antibody Deficiency. *J Allergy Clin Immunol,* 109**,** 581-91.

Baris, S., Karakoc-Aydiner, E., Ozen, A., Ozdemir, C., Bahceciler, N. N. & Barlan, I.B. 2011. Serum Immunoglobulin Levels As a Predictive Factor for a Better Outcome of Non-Atopic Childhood Asthma. *Pediatr Allergy Immunol,* 22**,** 298 304.

Buckley R. Evaluation of Suspected Immunodeficiency. In Nelson Textbook of Pediatrics. eds. Kliegman RM, Stanton BF,St Geme JW, Schor NF, Behrman RE, 19th ed. Philadelphia: Elsevier Saunders, 2011:715-722

Cave, A. J. & Atkinson, L. L. 2014. Asthma in Preschool Children: A Review Of The Diagnostic Challenges. *J Am Board Fam Med,* 27**,** 538-48.

De Freitas Dantas Gomes, E. L. & Costa, D. 2015. Evaluation of Functional, Autonomic and Inflammatory Outcomes in Children with Asthma. *World J Clin Cases,* 3**,** 301-9.

De Moraes Lui, C., Oliveira, L. C., Diogo, C. L., Kirschfink, M. & Grumach, A. S. 2002. Immunoglobulin G Subclass Concentrations and Infections in Children And Adolescents with Severe Asthma. *Pediatr Allergy Immunol,* 13**,** 195-202.

Delves, P. J., Martin, S. J., Burton, D. R. and Roitt, I. M. (2006). Essential Immunology. 11th ed. Italy: Blackwall Publishing.

Dogu, F., Ikinciogullari, A. & Babacan, E. 2004. Transient Hypogammaglobulinemia of Infancy and Early Childhood: Outcome of 30 Cases. *Turk J Pediatr,* 46**,** 120-4.

Dupin, C., Marchand-Adam, S., Favelle, O., Costes, R., Gatault, P., Diot, P., Grammatico-Guillon, L. & Guilleminault, L. 2016. Asthma and Hypogammaglobulinemia: An Asthma Phenotype with Low Type 2 Inflammation. *J Clin Immunol,* 36**,** 810-817.

Global Initiative for Asthma. Pocket Guide For Asthma Management and Prevention for Children 5 years and Younger [Internet]. 2015 [cited 2015 December 1]. Available from: http://www.ginasthma.org

Guilbert, T. W., Mauger, D. T. & Lemanske, R. F., Jr. 2014. Childhood Asthma Predictive Phenotype. *J Allergy Clin Immunol Pract,* 2**,** 664-70.

Hoeger, P. H., Niggemann, B. & Haeuser, G. 1994. Age Related Igg Subclass Concentrations in Asthma. *Arch Dis Child,* 70**,** 179-82.

Holgate, S. T. 2012. Innate and Adaptive Immune Responses in Asthma. *Nat Med,*18**,** 673-83.

Ikinciogullari, A., Kendirli, T., Dogu, F., Egin, Y., Reisli, I., Cin, S. & Babacan, E. 2004. Peripheral Blood Lymphocyte Subsets In Healthy Turkish Children. *Turk J Pediatr,* 46**,** 125-30.

Janeway, C. A., Travers, P., Walport, M. and Shlomchick, M. J. (2005). Immunobiology The Immune System in Health and Disease. 6th ed. New York: Garland Science Publishing.

Karaca, N. E., Karadeniz, C., Aksu, G. & Kutukculer, N. 2009. Clinical And Laboratory Evaluation of Periodically Monitored Turkish Children with Igg Subclass Deficiencies. *Asian Pac J Allergy Immunol,* 27**,** 43-8.

Karaman, O., Uguz, A. & Uzuner, N. 1999. Igg Subclasses In Wheezing Infants. *Indian J Pediatr,* 66**,** 345-9.

Keles, S., Artac, H., Kara, R., Gokturk, B., Ozen, A. & Reisli, I. 2010. Transient Hypogammaglobulinemia and Unclassified Hypogammaglobulinemia: 'Similarities And Differences'. *Pediatr Allergy Immunol,* 21**,** 843-51.

Lambrecht, B. N. & Hammad, H. 2015. The Immunology of Asthma. *Nat Immunol,* 16**,** 45-56.

Lasso-Pirot, A., Delgado-Villalta, S. & Spanier, A. J. 2015. Early Childhood Wheezers: Identifying Asthma in Later Life. *J Asthma Allergy,* 8**,** 63-73.

Male, D., Brostoff, J., Broth, D. B. and Roitt, I. (2006). Immunology. 7th ed. Philadelphia: Elsevier Ltd.

Martinez, F. D. 2002. What Have We Learned From the Tucson Children's Respiratory Study? *Paediatr Respir Rev,* 3**,** 193-7.

Martinez, F. D. & Vercelli, D. 2013. Asthma. *Lancet,* 382**,** 1360-72.

Moise, A., Nedelcu, F. D., Toader, M. A., Sora, S. M., Tica, A., Ferastraoaru, D. E. & Constantinescu, I. 2010. Primary Immunodeficiencies of the B Lymphocyte *J Med Life,* 3**,** 60-3.

Ober, C. & Yao, T. C. 2011. The Genetics of Asthma and Allergic Disease: A 21st Century Perspective. *Immunol Rev,* 242**,** 10-30.

Oner, A. F., Caksen, H., Celik, A., Cesur, Y., Uner, A. & Arslan, S. 2000. Serum Immunoglobulins and Immunoglobulin G Subclasses with Recurrent Wheezing. *Indian J Pediatr,* 67**,** 861-4.

Ones, U., Guler, N., Somer, A., Salman, N. & Yalcin, I. 1998. Low Immunoglobulin G3 Levels in Wheezy Children. *Acta Paediatr,* 87**,** 368-70.

Ozen, A., Baris, S., Karakoc-Aydiner, E., Ozdemir, C., Bahceciler, N. N. & Barlan,I.B. 2010. Outcome of Hypogammaglobulinemia in Children: Immunoglobulin Levels as Predictors. *Clin Immunol,* 137**,** 374-83.

Paul, M.E., Shearer, W.T. 1999. The child who has recurrent infection. Immunol Aller Clin North Am, 19;2;423-33.

Pieper, K., Grimbacher, B. & Eibel, H. 2013. B-Cell Biology and Development. *J Allergy Clin Immunol,* 131**,** 959-71.

Reddel, H. K., Bateman, E. D., Becker, A., Boulet, L. P., Cruz, A. A., Drazen, J. M., Haahtela, T., Hurd, S. S., Inoue, H., De Jongste, J. C., Lemanske, R. F., Jr., Levy,M. L., O'byrne, P. M., Paggiaro, P., Pedersen, S. E., Pizzichini, E., Soto Quiroz, M., Szefler, S. J., Wong, G. W. & Fitzgerald, J. M. 2015. A Summary of the New Gina Strategy: A Roadmap to Asthma Control. *Eur Respir J,* 46**,** 622 39.Sly, P. D., Boner, A. L., Bjorksten, B., Bush, A., Custovic, A., Eigenmann, P. A.,Gern, J. E., Gerritsen, J., Hamelmann, E., Helms, P. J., Lemanske, R. F., Martinez,

F., Pedersen, S., Renz, H., Sampson, H., Von Mutius, E., Wahn, U. & Holt, P. G. 2008. Early Identification of Atopy in the Prediction of Persistent Asthma In Children. *Lancet,* 372**,** 1100-6.

Spickett, G. (2013). Oxford Hanbook of Clinical Immunology and Allergy. 3rd ed. Oxford: Oxford University Press.

Stein, R. T. & Martinez, F. D. 2004. Asthma Phenotypes in Childhood: Lessons From An Epidemiological Approach. *Paediatr Respir Rev,* 5**,** 155-61.

Szczawinska-Poplonyk, A. 2012. An Overlapping Syndrome of Allergy and Immune Deficiency in Children. *J Allergy (Cairo),* 2012**,** 658279.

Van Aalderen, W. M. 2012. Childhood Asthma: Diagnosis and Treatment. *Scientifica (Cairo),* 2012**,** 674204.

Woroniecka M., Ballow M. 2000. Office evaluation of children with recurrent infection. Pediatr Clin North Am,47, 1211-24.