Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Kerbala
College of Medicine
Department of Microbiology

Respiratory bacterial infection influencing pediatric asthma: correlation to severity and control

A Thesis
Submitted to the Council of the College of Medicine/ University of Kerbala in Partial Fulfillments of the Requirements for the Degree of Master in Medical Microbiology

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2024 A.D

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صدق الله العلي العظيم

[سورة النساء (113)]

Supervisors Certification

We certify that this M.Sc. thesis titled:

"Respiratory bacterial infection influencing pediatric asthma: correlation to severity and control"

Was prepared under our supervision in the College of Medicine/ University of Kerbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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/ /2024

/ /2024

Committee certification

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"Respiratory bacterial infection influencing pediatric asthma: correlation to severity and control"

We have examined the student (**Mustafa Qahtan Hashem Mohsen**) in its contents. In our opinion it is meets the standards of thesis for the degree of Masters in Medical Microbiology and Immunology.

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Dedication

To the inspiration of humanity, Great Prophet …Mohammad and Ahlu albeit

To whom I carry his name proudly, the greatest man …....My Father

To my angel in life, whose prayer was the secret of my success... The most precious woman in my life, My …Mother

To the one who taught me the meaning of loyalty and devotion, and bore with me the burdens of life, with its happiness and misery... my wife is the secret to my ….peace of mind

To my brothers, friends and everyone who helped me

To my lofty country (Iraq)

Mustafa 2024

ACKNOWLEDGMENTS

I would like to thank Almighty God for helping me to work in fulfilling the requirements of this research.

I would like to thank the Department of Microbiology, College of Medicine, University of Karbala for providing all the facilities and guidance.

I would like to express my deepest gratitude and sincere thanks to my supervisors. Professor Dr. Sawsan M. Jabbar AL-Hasnawi and Assistant Professor Dr. . Ashwaq Ali Hussein for their great interest, kind support and invaluable advice.

Thanks and appreciation go to Dr. Maitham Al-Maamouri, and Dr. Hussam Abbas Al-Dhalimi, Asthma and Allergy Specialist

All thanks and gratitude go to asthmatic children and their parents of their contribution to the study.

Mustafa 2024

Summary:

Asthma is a chronic inflammatory disorder of the airways characterized by recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. Respiratory infections significantly impact the management and prognosis of pediatric asthma, often triggering exacerbations and complicating treatment regimens. This study aims to evaluate the association between bacterial respiratory infection with asthma severity, and control.

This is a cross-sectional study analyzed data from 100 asthma patients aged 5-16 years at the karbala hospital for teaching children, And AI- Imam AL-Hussein Teaching Medical City. The data were collected from first of October \2023\ to 30 Th of May (2024). Sample Collection contained sputum and cough samples collected after patients rinsed their mouths with saline or water and samples were transported immediately to the laboratory. To identify the bacteria, they are inoculated on a variety of media (aerobic) including trypticase soy agar, 5% sheep blood agar, Mac Con Key agar, and The Mannitol salt agar. The cultures were then incubated for a full night at 37°C; if no growth is seen, the cultures ware reincubated for a further 24 hours and the VITEK2 compact system was used to identify the bacterial isolates. For statistical analysis, data were loaded into the statistical suite for the Social Sciences (SPSS). The results of this study showed the majority (57%) of asthma patients were aged 5-8 years, with significant differences noted (p=0.0001). A significant majority were male (69%) compared to female (31%) (p=0.0001). Clinical Characteristics of Asthma Patients: A history of COVID-19 infection was negative in 85%. A large percentage of patients (77%) had comorbidities such as sinusitis, rhinitis & gastroesophageal reflux(p=0.0001) with 95% having been using inhaled corticosteroids for more than 3 years.

Bacterial Culture Results: *Streptococcus pneumoniaee* was the most isolated pathogen (11%). Significant differences in lymphocytesocyte and total IgE levels based on bacterial growth, with the highest concentrations seen in patients with *Staphylococcus aureus*. There was also a high significant association between poor asthma control and the bacterial isolates of *Klebsiella pneumoniaeStreptococcus pneumoniae*and *Pseudomonas aeruginosa*.

The present study concluded Gram-negative bacteria were more prominent among respiratory tract infections. *Streptococcus pneumoniaee* was the most common bacterial isolate. Also a significant association between poor asthma control and the presence of specific bacterial isolates. Laboratory marker as lymphocyte and immunoglobulin E (IgE) was affected by the type of bacterial growth, especially in those with *Staphylococcus aureus* infections, which may indicate an immune response to these pathogens.

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List of abbreviations

Code	word
ABa	**Allergic bronchial asthma**
AD	**allergic diseases**
AHR	**Allergic hyperresponsiveness**
AIT	**Allergy immunotherapy**
APCs	**Antigen presenting cells**
AUC	**area under the curve**
BA	**Bronchial asthma**
BHR	**bronchial hyperresponsiveness**
BAL	Bronchoalveolar lavage
ABPA	**bronchopulmonary aspergillosis**
CS	**Caesarian section**
COPD	**Chronic obstructive pulmonary disease**
CBC	**Complete blood count**
DC	**dendritic cell**
ED	**emergency department**
(EIA)	**enzyme immunoassay**
ELISA	**Enzyme-Linked Immunosorbent Assay**
EBC	**eosinophilophil blood count**
EAACI	**European Academy of Allergy and Clinical Immunology**
FH	family history
FEV1	**Forced vital capacity**
FVC	**Forced vital capacity**
FeNo	**fractional exhaled nitric oxide**
GINA	**Global Initiative**
GNB	**Gram-negative bacteria**
GM- CSF	**granulocyte-macrophage colony-stimulating factor**
GM	**gut microbiota**
HDM	**house dust mites**
IgE	**Immunoglobulin E**
ICS	**inhaled cortecosteroid**
LPS	**lipopolysaccharide**
LPS	**lipopolysaccharides**
MBP	**major binding protein**

MSA	**Mannitol Salt Agar**
MDR	**multidrug-resistant**
NK	**natural killer**
NPV	**negative predictive value**
NA	**neutrophilic asthma**
NLR	neutrophil-lymphocytesocyte ratio
N	**number URT Upper respiratory tract**
OD	**optical density**
OPS	**Oropharngeal swab**
PA	**Pediatric Asthma**
PPV	**Positive predictive value**
RV	**Respiratory syncytial virus**
RTIs	**Respiratory tract infection**
SA	**Severe asthma**
SES	**socioeconomic status**
Std	**Stander deviation**
TAs	**teichoic acids**
TH	**T-helper cells**
TSLP	**thymic stromal lymphocytesopoietin**
TLR	**Toll-like Receptors**
TNF	**tumour necrosis factor**
TNF	**tumour necrosis factor**
URT	**upper respiratory tract**

Chapter one

Introduction & literature Review

1.1Introduction

Asthma is a complex disorder characterized by variable recurrent symptoms, airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation and remodelling(Carr, Zeki & Kraft, 2018.Papadopoulos *et al.*, 2024)

Pathological features of the airway in children with severe recurrent wheeze suggest an association between bacterial colonization and the initiating events of early asthma(Bisgaard *et al.*, 2007)

Asthma often starts at a young age (childhood-onset asthma), but some patients can develop asthma later in life (late-onset asthma). Childhood-onset and late-onset asthma differ in many ways. Late-onset asthma is more severe and less associated with allergy than childhoodonset asthma. In children, atopy, , and respiratory- tract infections especially with rhinovirus, represent major risk factors for the persistence of asthma(Hammad & Lambrecht, 2021)

It is believed that several inflammatory endotypes, impacted by numerous hereditary and environmental variables, are the cause of asthma. Because of its complexity, developing preventative and disease-modifying medicines for asthma has been difficult, and there is still a clinical need that is unmet.(Ackland *et al.*, 2021)

Bacteria clearly have roles to play in the disease progress and clinical outcome of asthma but also appear, in certain cases, to play protective functions(Earl, An & Ryan, 2015)

Airway neutrophilia has been associated with asthma severity and asthma exacerbations; however, neutrophils can also be detected in the airways of both healthy subjects and mild asthmatics.also Asthma has long

been associated with eosinophilophilic inflammation as well as IgE-mediated mast cell activation, commonly described as a component of the ‘atopic march (Ray & Kolls, 2017)

The split among eosinophilophilic and non-eosinophilophilic subphenotypes of asthma relies on changes in cells active in respiratory airway inflammation. Eosinophile have recently been found in the airways of nonallergic (intrinsic) asthma. formerly, eosinophilophilic inflammation has been associated with extrinsic (allergic) asthma with Th2-type response.(Carr, Zeki & Kraft, 2018)

The relationship between asthma and infections is complex and involves the pathogen and factors associated with the host genetic background(Fraga-Silva *et al.*, 2023)

In children asthma patients, eosinophilophilic inflammation is usually seen in mild-to-moderate disease and neutrophilic inflammation in more severe disease(Vroman, van den Blink & Kool, 2015)

The airways have an array of microbes. Promoting and maintaining immunological tolerance and preventing an unwanted, unpredictable inflammatory response put on by inhaling innocuous environmental stimuli are two new roles for lung bacteria. This impact is accomplished by a constant relationship between immune cells present in the lungs and commensal bacteria, which express a variety of sensors used to identify microorganisms. The pathogen identifying process and the creation of a healthy immune response are both mediated by the same receptors. due its crucial function in maintaining lung homeostasis, the lung microbiota may also be viewed as a sign of the state of lung health.(Sommariva *et al.*, 2020)

The hygiene hypothesis consider that repeated exposure to diverse common infections (in particular, with bacteria and exposure to

environmental microbiota during childhood are strongly associated with a healthy maturation of the immune system and with protection from the development of asthma and allergies later in life(Pelaia *et al.*, 2015)

Aim of the study

: The goal of the study is to evaluate the association between bacterial respiratory infection and asthma severity, type, and control through the following objectives:

1-Identify the type of bacterial infection in the respiratory tract of children.

2-Correlate specific bacterial growth with some blood tests as serum total IgE and CBC.

3-Correlate specific bacterial growth with asthma severity, type, and control.

1.2. Literature review

1.2.1. Asthma overview

Asthma is a future lung disorder characterized by bouts of dyspnea brought on by constricted airways. A hyperreactive immune response to harmless allergens causes the condition, which then manifests as hyperinflammation, mucus formation, alterations in the structural cells lining the airways, and hyperresponsiveness of the airways. Even though it affects adults, asthma tends to be thought of as a childhood illness, affecting over 6.2 million children annually under the age of 18.(Mthembu *et al.*, 2021)

Asthma is a consequence of complex gene–environment interactions, with heterogeneity in clinical presentation and the type and intensity of airway inflammation and remodelling(Kim *et al.*, 2021)

Few studies have been conducted to determine the prevalence of asthma among Iraqi children, as the results of the studies showed that the prevalence of asthma varies according to geographical regions in the country. The highest rates of asthma were in the capital, Baghdad, and the lowest in the far north, in Erbil Governorate. there is a need to conduct a new study to determine the prevalence of asthma in Iraq, determine financial allocations, and address the resulting environmental problems that would interfere with the treatment method and quality of life for asthma patients.(Alsajri, Al-Qerem & Mohamed Noor, 2023)

As can be seen from the collected data, asthma symptoms could begin during early childhood. Most asthmatic children continue to have an atopic constitution with sensitivity to specific allergens being a significant risk factor(Lee, Yang & Hwang, 2024)

Asthma and pneumoniae are common respiratory conditions globally,

affecting individuals of all ages. *Streptococcus pneumoniaee* is the predominant bacterial cause of pneumoniae, with nasopharyngeal carriage an important step towards invasive and pulmonary disease(Zaidi & Blakey, 2019)

Infections with atypical bacteria also appear to play a role in the induction and exacerbation of asthma in both children and adults(Webley & Hahn, 2017)

1.2.2.Epidemiology and Prevalence of Asthma in children

One of the most prevalent chronic diseases in the world, asthma affects about 300 million individuals worldwide While the disease is well-known throughout the world,(Prakash, 2024), and it is predicted that by 2025, an additional 100 million people may be affected(Paramonova *et al.*, 2024)

Asthma is one of the most common and costly chronic diseases in the United States (US). A total of 25 million Americans have asthma; of these, 20.2 million are adults(Swed *et al.*, 2024)

Asthma is extremely common with a prevalence of approximately 10% in Europe. It presents with symptoms which have a broad differential diagnosis and examination can be entirely normal(Räisänen *et al.*, 2021)

In Asia-Pacific The prevalence rates of asthma and allergic rhinitis appear to have recently reached a plateau in Western countries, whereas they are still increasing in many Asian countries. Given the large population in Asia, even a slight increase in the prevalence rate will translate into an overwhelming number of patients.(Xing & Wong, 2022)

Asthma control in the Middle East and North Africa is unsatisfactory with less than one-third of asthma patients having controlled disease, highlighting the need to improve treatment access and medication

adherence, along with better follow-up and education among healthcare providers and patients.(Tarraf, *et al.*, 2018)

The observed adjusted prevalence of asthma in the Middle East ranges from 4.4% to 7.6%, which is comparatively lower than the reported prevalence in Europe and North America. Asthma has a negative impact on quality of life, and is associated with high levels of co-morbid diseases,(Tarraf, *et al.*, 2018)

Prevalence of asthma in the Middle Eastern children aged 13-14 years was 7.57% (95% Cl: 6.38-8.75). The minimum prevalence rate of asthma (0.7%) was observed in Isfahan in Iran, and the highest 22.3% was reported from Bagdad in Iraq(Mirzaei *et al.*, 2017).

In Iraq, about 200,000 patients per year with asthma are either hospitalised or treated in an emergency room(Hamdan, Al-Attar & Hashim, 2019)

Few studies have been conducted to determine the prevalence of asthma among Iraqi children, as the results of the studies showed that the prevalence of asthma varies according to geographical regions in the country. The highest rates of asthma were in the capital, Baghdad, and the lowest in the far north, in Erbil Governorate. Most of the published studies date back to ancient history, and there is a need to conduct a new study to determine the prevalence of asthma in Iraq, determine financial allocations, and address the resulting environmental problems that would interfere with the treatment method and quality of life for asthma patients(Alsajri, Al-Qerem & Mohamed Noor, 2023).

Asthma is the the most common chronic respiratory condition of childhood worldwide, with around 14% of children and young people affected. Despite the high prevalence, paediatric asthma outcomes are inadequate, and there are several avoidable deaths each year.(Papi *et al.*, 2018);(Martin,

Townshend and Brodlie, 2022).

While a variety of variables, including food allergies, antibiotic use, birth type, genetics, and exposure to cigarette smoke, can impact the development of asthma, in particular for children, respiratory infections are considered to be the primary cause of poor lung function and the onset of the illness.(Mthembu *et al.*, 2021).

Asthma affected an estimated 262 million people in 2019 and caused 455 000 deaths (Kim *et al.*, 2023).

The prevalence of asthma has increased; however, the number of patients who die from it has decreased (1.3 per 100,000 patients in 2018).(Nakamura *et al.*, 2020).

Patients with severe asthma (SA) have a heightened risk of exacerbations including hospitalization(Trevor *et al.*, 2021).

Asthma has become more common in adults and children as of the second half of the 20th century.The frequency of asthma in childhood and adulthood has an impact on the prevalence in adults.(Räisänen *et al.*, 2021);(Räisänen *et al.*, 2024).

Males and females through different ages experience it at times and of variable intensity. Asthma is more common in guys when they are young. Asthma severity and prevalence are higher in women as adults.(Chowdhury *et al.*, 2021).

During the past 50 years, the prevalence of asthma has increased and this has full to gether with our changing relation with microorganisms(Earl, An & Ryan, 2015).

1.2.3 Asthma Exacerbation

Acute severe asthma exacerbations can very rapidly evolve into near fatal asthma, and it is imperative that the critical care team makes a swift and thorough assessment of all referred patients(Talbot, Roe and Dushianthan, 2024);Gayen *et al.*, 2024).

Acute asthma attacks are increasing episodes of shortness of breath, cough, wheezing or chest tightness associated with a decrease in airflow that can be quantified and monitored by measurement of lung function (peak expiratory flow (PEF) or forced expiratory volume in the 1st second) and requiring emergency room treatment or admission to hospital for acute asthma and/or systemic glucocorticosteroids for management(Lalloo *et al.*, 2013).

Airway colonisation with potentially pathogenic micro-organisms in asthma is associated with more severe airways obstruction and neutrophilic airway inflammation. This altered colonisation may have a role in the development of an asthma phenotype that responds less well to current asthma therapies (Crisford, Sapey, Geraint B. Rogers, *et al.*, 2021).

Including near-fatal asthma (NFA), have high morbidity and mortality. Mechanical ventilation of patients with severe asthma is difficult due to the complex pathophysiology resulting from severe bronchospasm traditional ventilation strategies in asthma exacerbations include the development of systemic hypotension from hyperinflation, air trapping, and pneutrophilmothoraces(Gayen *et al.*, 2024).

The excess presence of bacteria, has also been identified in association with wheezing and acute asthma exacerbations, including *haemophilus influenza, streptococcus pneumoniaemoraxella -catarrhalis, mycoplasma pneumoniae and chlamydophila pneumoniae*(Papadopoulos

et al., 2024).

A major part of the burden of asthma is caused by acute exacerbations. Exacerbations have been strongly and consistently associated with respiratory infections. Respiratory viruses and bacteria are therefore possible treatment targets. To have a reasonable estimate of the burden of disease induced by such infectious agents on asthmatic patients, it is necessary to understand their nature and be able to identify them in clinical samples by employing accurate and sensitive methodologies(Papadopoulos *et al.*, 2011;Papadopoulos *et al.*, 2024).

Bacterial colonization of the lower airways is common in patients with chronic severe asthma and is linked to the duration of asthma and having had exacerbations in the past year, but not with an increase in airway wall thickness(Zhang *et al.*, 2012).

1.2.4 Asthma Severity

Severe asthma is associated with an increased risk for exacerbations, reduced lung function, fixed airflow obstruction, and substantial morbidity and mortality(Farinha and Heaney, 2024).

Severe asthma (SA) is a refractory condition that does not respond well to conventional treatments. Patients with SA have heterogenetic endotypes. Endotypes for SA can be classified as type 2 cytokine-high and type 2 cytokine-low. The condition can also be classified as eosinophilophilic, neutrophilic, mixed, and paucigranulocytic SA. Abnormalities in TH1 and TH17 cytokines can be present in some SA patients. Innate lymphocytesoid cells, airway smooth muscle cells, and lung epithelial cells have important roles in the disease. Viral infections, bacterial infections, fungal infections, smoking, allergens, and pollutants are major triggers that determine the severity of the disease.(Du *et al.*, 2024).

Asthma severity (defined as a change in Asthma Control Test score of at least 3 points and a change in Global Initiative for Asthma classification from uncontrolled or partially controlled to well-controlled) and those with no improvement or worsening of asthma severity(Khan *et al.*, 2024).

According to the current state of clinical control and associated risks, severe asthma is defined as "uncontrolled asthma which can result in risk of frequent severe exacerbations (or death) and/or adverse medication reactions and/or chronic morbidity (including reduced lung growth or function in children)." Three categories of severe asthma exist, each with its own set of public health messages and challenges: treatment-resistant severe asthma, which includes asthma for which control is not achieved despite the highest level of recommended treatment or asthma for which control is only achieved with the highest level of recommended treatment. Untreated severe asthma is caused by undiagnosed asthma or lack of therapy. Difficult-to-treat severe asthma is caused by adherence issues, inappropriate or incorrect medication use, environmental triggers, or co-morbidity.(Bush and Zar, 2011).

Severe asthma affects 3.6–10.0% of patients with asthma, which corresponds to around 4 million patients globally. Currently, much research is focused on pathomechanisms of severe asthma and development of its new biological therapies. Although it is much less prevalent than mild and moderate asthma, severe asthma contributes to about 60% of costs associated with this disease, mainly due to drug costs(Kardas, Kuna and Panek, 2020) ;(Kardas, Kuna and Panek, 2020) ;(Hussain and Liu, 2024).

Severe asthma in children is associated with significant morbidity(Haktanir Abul and Phipatanakul, 2019).

In severe asthma, there is an unclear distinction between T (type) 2 high

and T2 low airway inflammation. The production of important cytokines, interleukin (IL)-4, -5, and -13, which cause and control airway inflammation, is a hallmark of T2 high. Eosinophile, fractional exhaled nitric oxide (FeNO), and immunoglobulin (Ig) E—whose Being results from the interaction of IgE, IL-5, IL-4, and IL-13—are biomarkers that signify the presence of T2-high inflammation. These drugs target the pathways that cause inflammation. The available monoclonal antibodies include omalizumab (anti-IgE); mepolizumab, reslizumab and benralizumab (anti-IL-5 pathways), and dupilumab (anti-IL-4/IL-13).(Busse, 2019).

More research is required to determine the significance of environmental exposures in the development of asthma in both children and adults, even though many significant environmental factors that induce asthma are well-established. There is mounting evidence that exploring potential connections between genes and environments as well as environments and genes helps to identify the factors that influence asthma. (Peters, Dixon and Forno, 2018)(Dharmage, Perret and Custovic, 2019).

Allergic sensitization is an important factor in the development, severity, and exacerbation of asthma, which is attributed to type 2 (Th2) inflammation. Evidence suggests that respiratory bacterial pathogens (e.g., *Streptococcus pneumoniaee*) exert suppressive effects on airway Th2 inflammation(Kama *et al.*, 2022).

Moreover, moving from severity-based to phenotype-based asthma care can improve the care of asthma and allergic diseases. Timely diagnosis of aeroallergen sensitizations forms the basis for individualized treatment plans, which may include allergen remediation strategies when appropriate,

and allergen immunotherapy, the only immunomodulating therapy for allergic asthma(Casale *et al.*, 2020).

1.2.5 Pathogenesis

A chronic inflammatory illness of the airways, asthma causes coughing, wheezing, shortness of breath, and heaviness in the chest. The inflammation of the airways causes the creation of mucus, remodeling of the airway wall, and bronchial hyperresponsiveness (BHR), which is the smooth muscle cells' propensity to respond to non-specific stimuli like cold air. These processes are what cause the symptoms of asthma. Some people can develop asthma later in life (late-onset asthma and late-onset asthma are more common), but asthma frequently begins early in life (childhood-onset asthma). More severe and less related to allergies than asthma that starts in childhood(Hammad and Lambrecht, 2021).

Bronchial asthma is a heterogeneous disease characterised by chronic airway inflammation. A variety of immune cells such as eosinophile, mast cells, T lymphocyte, neutrophils and airway epithelial cells are involved in the airway inflammation and airway hyperresponsiveness in asthma pathogenesis, resulting in extensive and variable reversible expiratory airflow limitation(Kulkarni & Kediya, 2022).

Asthma is divided into 4 inflammatory subtypes: eosinophilophilic asthma, neutrophilic asthma (NA), mixed granulocytic asthma, and paucigranulocytic asthma (Yang *et al.*, 2018).

Airway hyperresponsiveness (AHR), epithelial cell activation, mucus overproduction, and airway remodeling are all linked to asthma. Innate immunity and adaptive immunity are involved in the immunologic pathways underlying asthma. One prevalent asthmatic phenotype is type 2 asthma with eosinophilophilia. It happens both when an allergy is obvious

and not. The type 2 endotype is made up of eosinophile, type 2 innate lymphocytesoid cells (ILC2), B cells that secrete IgE, and T helper type 2 (Th2) cells. ILC2 predominates in eosinophilophilic nonallergic asthma, which generates IL-5 to draw eosinophile into the mucosal airway. Non-type 2 asthma, which includes a diverse range of endoypes and phenotypes like exercise-induced asthma, obesity-induced asthma, etc., is the second main subgroup of asthma. Allergens do not cause neutrophilic asthma; instead, infections and cigarette smoke can(Frey *et al.*, 2020).

A detrimental shift in the balance between beneficial and detrimental lifestyle choices and environmental factors, such as exposure to beneficial commensal microbes versus pathogen infection, is thought to facilitate the onset of disease and cause damage to airway epithelial cells and disruption of the barrier integrity. One of the primary triggers for the type 2 immune response to harmless allergens is the release of cytokines from epithelial cells. These cytokines eventually cause type 2 T helper (TH2) cells, type 2 innate lymphocytesoid cells (ILC2s), M2 macrophages, and eosinophile to infiltrate lung tissue.(Komlósi *et al.*, 2022).

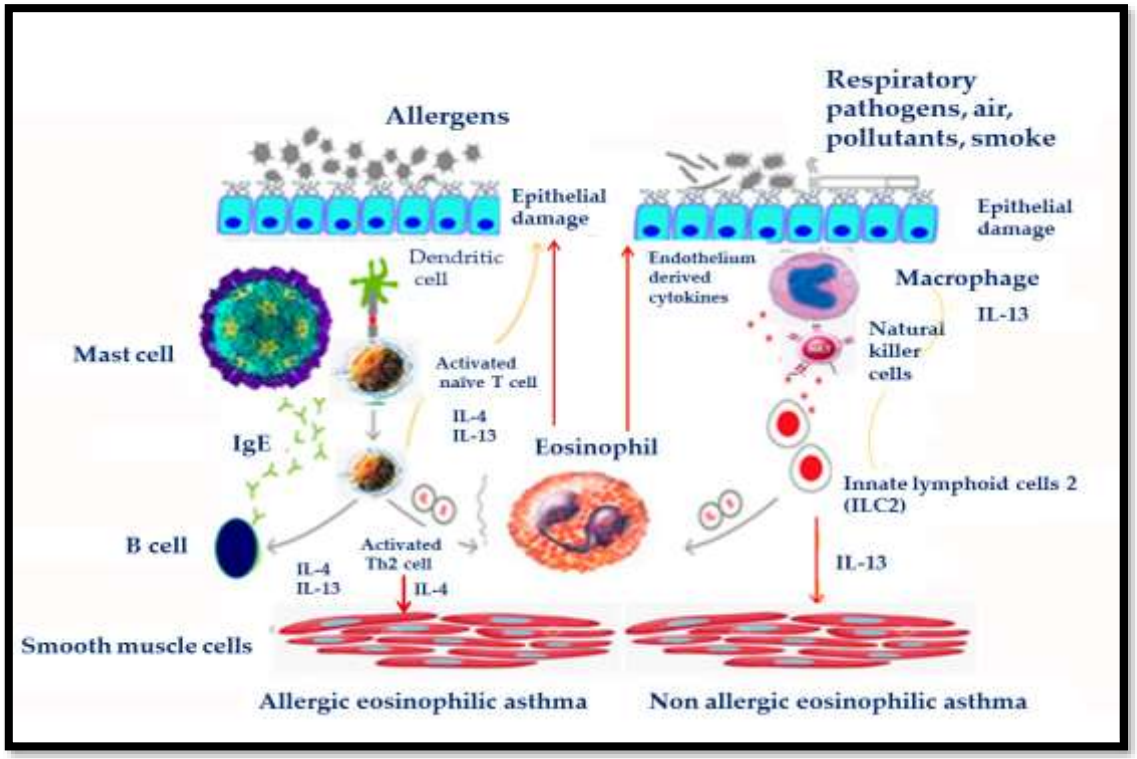
Asthma is divided into two categories: intrinsic and extrinsic. Commonly referred to as allergic asthma, extrinsic asthma is triggered by allergens and mostly associated with aberrant Th2 (Th2) inflammation. Numerous things, including aspirin, lung infections, exercise, cold, stress, and obesity, can cause intrinsic asthma. (Habib, Pasha & Tang, 2022).

The three main characteristics of allergic asthma are mast cell activation, eosinophilophilic infiltration, and IgE production. Asthma caused by allergies is associated with atopic dermatitis and allergy-related rhinitis. It usually appears in childhood. Non-allergic asthma may present with a late onset of TH2-high immune response with eosinophilophilic infiltration or TH2-low immune response. The TH2-low endotype's distinctive

neutrophilic inflammation can be triggered by infections, exposure to environmental irritants (such as diesel particles and cigarette smoke), and obesity.. (Fraga-Silva *et al.*, 2023).

Eosinophilophilic asthma, a subtype of asthma, involves the accumulation of eosinophile in the airways. These eosinophile release mediators and cytokines, contributing to severe airway inflammation and tissue damage. Chronic inflammation can induce structural changes in the airways, resulting in airway remodeling characterized by thickening of the smooth muscle and increased mucus production. Furthermore, bronchial hyperresponsiveness, a heightened sensitivity of the airways to stimuli, contributes to exaggerated bronchoconstriction and airflow limitation. (Hussain & Liu, 2024).

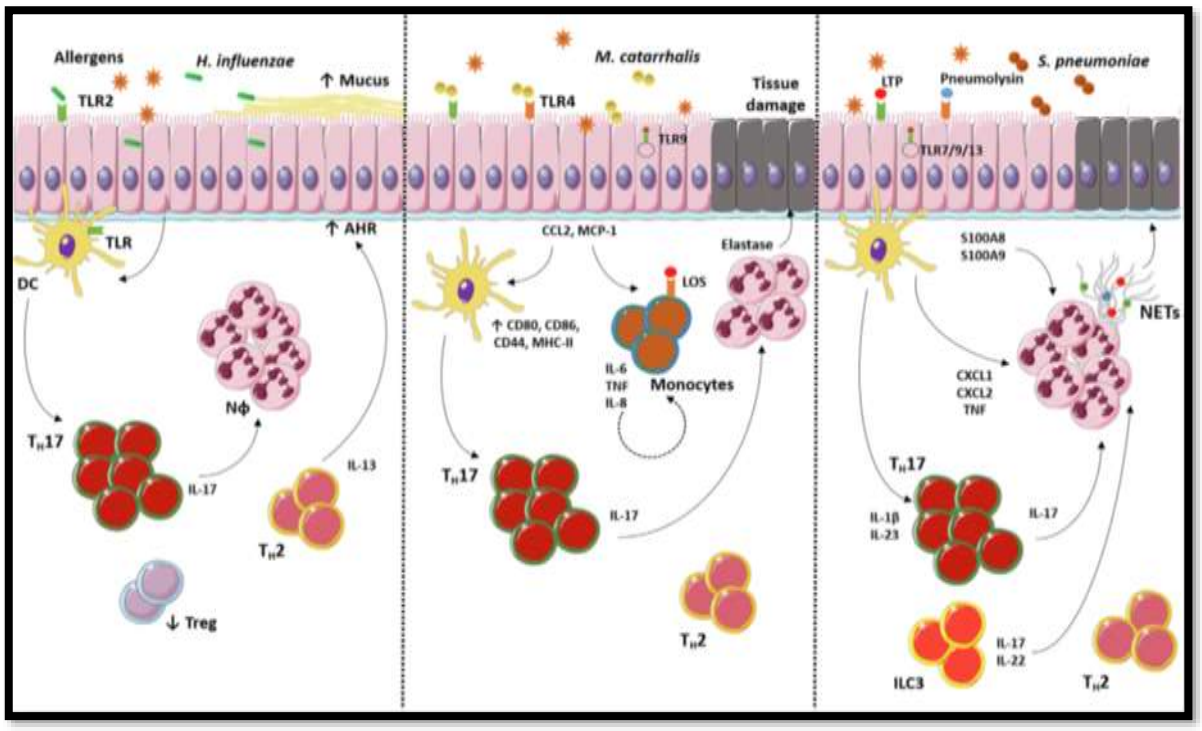
Inflammatory phenotypes of severe asthma caused by eosinophilophilic infiltration are identified by the persistence of either neutrophilic or eosinophilophilic infiltration in addition to the lack of paucigranulocytic (inflammatory) infiltration. Asthma endotypes are classified as either non-type 2 asthma, linked to Th1 and/or Th17 cell inflammation, or type 2 asthma, defined mostly by T helper type 2 (Th2) cell-mediated inflammation, depending on the kind of immune cell responses engaged in disease etiology. About 50% of persons with asthma have eosinophilophilic, Th2 airway inflammation, and this percentage is thought to increase in the absence of corticosteroids.(Kostakou *et al.*, 2019).



Figure(1.1) . Pathogenesis of acute exacerbations in asthma.(Kostakou *et al.*, 2019)

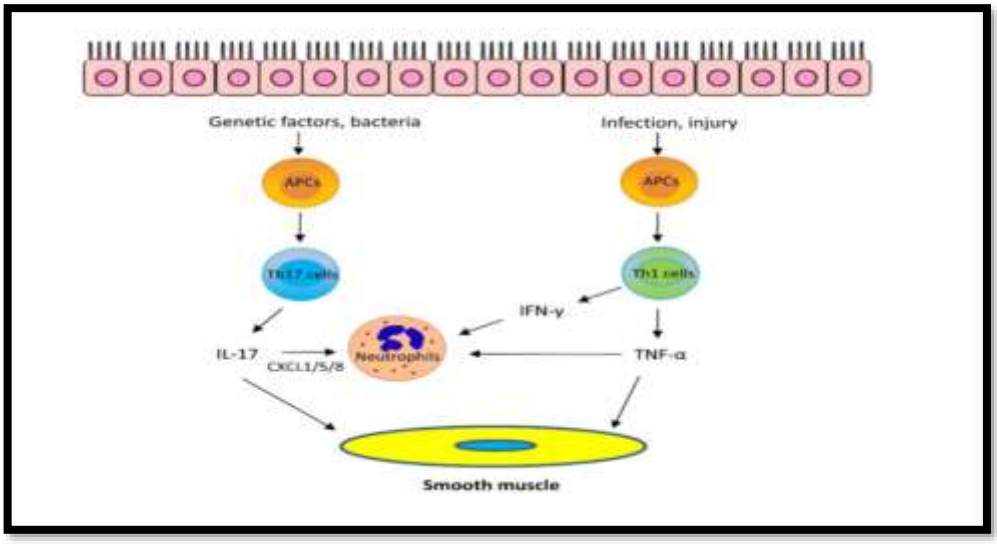
Th2-high and Th2-low asthma are the two categories into which the condition has been divided according to the level of Th2 inflammation. While neutrophilic asthma and paucigranulocytic asthma are included in Th2-low asthma, eosinophilophilic airway inflammation, which is linked to elevated blood eosinophilophil counts or fractional exhaled nitric oxide (FeNo), is the hallmark of Th2-high asthma.(Habib, Pasha and Tang, 2022).

The intricate relationship between infection by bacteria and asthma encompasses both pathogen-specific immune responses and asthma. In people with allergic asthma (eosinophilophil inflammation), the comorbidities may impact the lung milieu and amplify a preexisting TH2 profile; in severe asthma cases, they may trigger the immune response to a TH17 profile and result in neutrophil or granulocytic inflammation. Certain infections may cause TH17 cells to become activated, which therefore facilitates neutrophil inflow into the airways and aggravates asthma..(Fraga-Silva *et al.*, 2023).



Figure(1.2).Bacterial infections and asthma exacerbation(Fraga-Silva *et al.*, 2023).

Neutrophilic asthma (NA) is an important asthma inflammatory phenotype associated with disease severity, airflow limitation, and steroid resistance, that neutrophilic inflammatory phenotype in asthma is associated with increased bacterial burden in airways, along with a disordered airway microbiome and excessive airway inflammation. Airway dysbiosis is one of the key factors underlying the heterogeneity of asthma inflammatory phenotypes.(Yang *et al.*, 2018)



Figure(1.3).The Th2-high asthma mechanism

Dendritic cells (DCs) deliver allergens to Th2 cells when they enter the low airways. Th2 cells then generate Th2 cytokines, such as interleukin (IL)-5, IL-4, and IL-13. B cells are stimulated by IL-4 and IL-13 to generate IgE. After that, IgE attaches itself to the mast cell surface. The same allergens interact with IgE as they enter the airways, causing mast cells to release mediators such histamine, leukotrienes (LTs), and ILs. These mediators cause bronchoconstriction by irritating smooth muscle in the airways. Furthermore, IL-5 promotes the recruitment of eosinophile into the lungs. Additionally, mast cells are stimulated to release histamines and LTs by eosinophile through the secretion of mediators such as major basic protein (MBP). In addition, MBP causes bronchospasm, inhibits the M2 receptor, and encourages the release of acetylcholine from cholinergic neutrophilrons. Moreover, IL-13 directly increases(Habib, Pasha & Tang, 2022).

The pathogenic role of immunoglobulin E (IgE) antibodies in triggering and maintaining allergic inflammation in response to allergens is due to the binding of multivalent allergens to allergen-specific IgEs on sensitized effector cells. These interactions trigger effector cell activation, resulting in release of potent inflammatory mediators, recruitment of inflammatory cells, antigen presentation, and production of allergen-specific antibody responses(Karagiannis *et al.*, 2013) , the immune cells coated with IgE

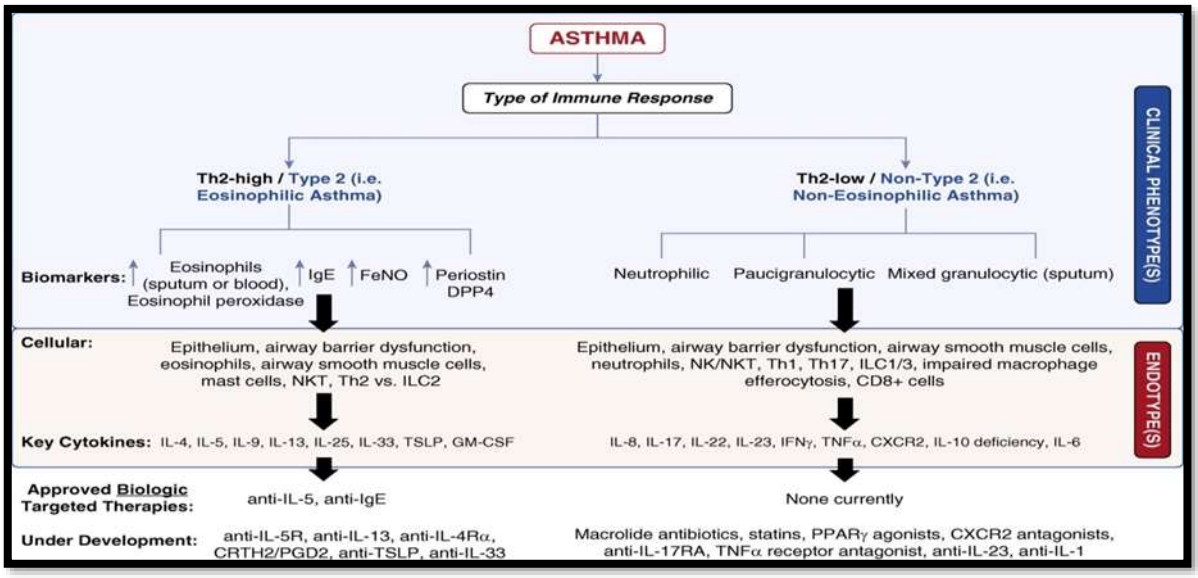
release chemicals that cause inflammation and swelling, and those chemicals are the cause of the itching and sneezing symptoms that often occur in the body's attempt to remove the allergen. The Th2 cells can also go to the site where the allergen entered the body and promote inflammation in that site. For example, the eyes of an allergic person can become swollen, red, and itchy in response to an allergen in the air, like pollen (De *et al.*, no date)

Allergic asthma is defined as asthma associated with sensitization to aeroallergens, which leads to asthma symptoms and airway inflammation. Allergic asthma is the most common asthma phenotype. The onset of allergic asthma is most often in childhood and is usually accompanied by other comorbidities including atopic dermatitis and allergic rhinitis(Fraga-Silva *et al.*, 2023)

Allergic asthma is the most common asthma phenotype. It usually is defined by the presence of sensitization to environmental allergens, although a clinical correlation between exposure and symptoms further supports the diagnosis(Vera, 2016).

Despite the presence of a variety of natural commensals and an opportunistic pathogenic microbiome in the human upper airway, an imbalance in these interactions results in pathogen overgrowth, increased inflammation, and airway remodeling. The pattern of this inflammation may be determined by competition for epithelial cell attachment, varying susceptibilities to antimicrobial peptides and host defense molecules, generation of proinflammatory cytokines, and pattern recognition receptors. Infection, air pollution, smoking, and other environmental variables are frequently linked to asthma comorbidity, severity, exacerbation, and resistance to antimicrobial and steroid treatment. Host and microbial genetics may also influence these outcomes(Losol *et al.*, 2021)

In adult refractory asthma patients, prolonged airflow restriction has been linked to airway neutrophilia. The recruitment and activation of neutrophils into the airways, which causes a shift toward Th1 and Th17 responses, has been connected to the activation of innate immunity and toll-like receptor signaling. This process leads in increased synthesis of interleukin (IL)-8, IL-17A, neutrophil elastase, and a kind of matrix metalloproteinase-9 (MMP-9) that is less inhibited by tissue inhibitors of metalloproteinases. In adult patients with severe neutrophilic asthma, these cytokines and activated enzymes can change the architecture of the airways, which can result in remodeling, fixed airway obstruction, and a decreased forced exhalation volume(Froidure *et al.*, 2016; Jusufovic, 2022;Fraga-Silva *et al.*, 2023; Versi *et al.*, 2023)



Figure(1.4).Clinical phenotypes, endotypes, and therapeutic options in eosinophilophilic and noneosinophilophilic asthma(Carr, Zeki and Kraft, 2018;Fraga-Silva et al., 2023)

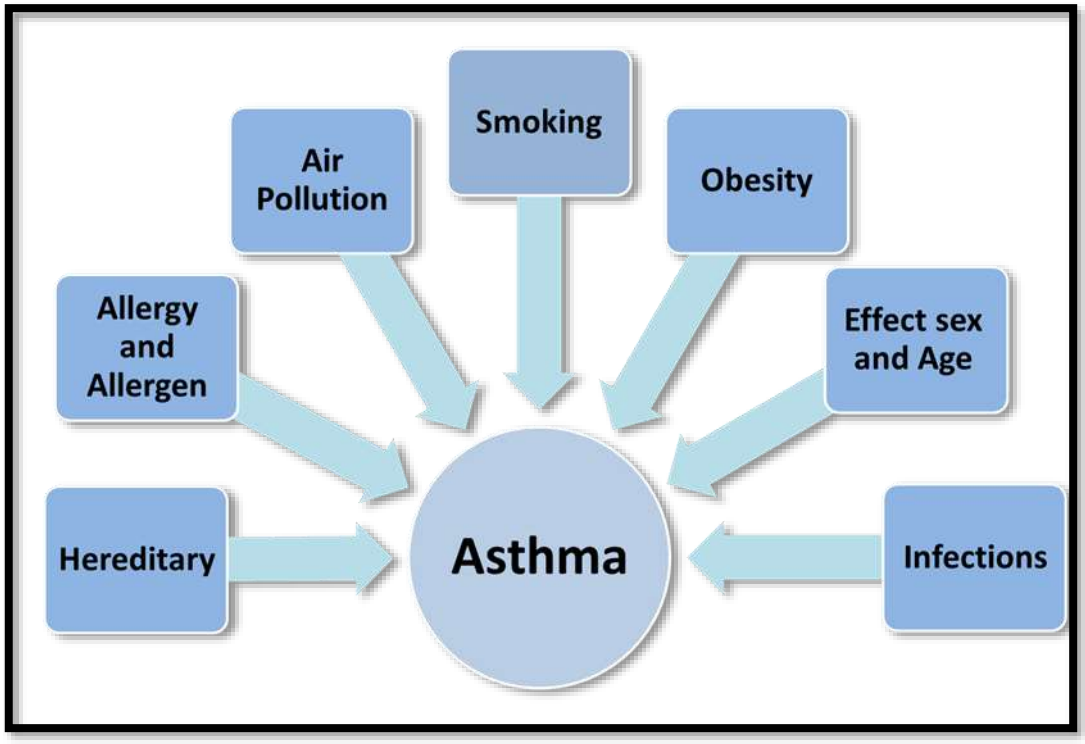
Asthma is a phenotypically heterogeneous chronic disease of the airways, characterized by either predominant eosinophilophilic or neutrophilic, or even mixed eosinophilophilic/neutrophilic inflammatory patterns(Pelaia *et al.*, 2015)

Bacteria and their metabolites are responsible for molecular alterations in the host's immune system; they can affect cell differentiation as well as modulate cell responses and the secretion of proinflammatory or protective factors, such as cytokines, interleukins, or antibodies. Microbial dysbiosis may lead to inflammatory system disturbances and contribute to the development of allergic diseases, especially asthma, by promoting opportunistic pathogen invasion caused by imbalanced immune responses. (Loverdos *et al.*, 2019;Jartti *et al.*, 2020; Logoń *et al.*, 2023)

1.2. 6 Risk Factors of Asthma

The causes of asthma are multifactorial and include a complex mix of environmental, immunological and host genetic factors (Liu *et al.*, 2020;Y. Li *et al.*, 2023).

The development of asthma is complex and is likely to be influenced by genetics and environment factors in utero and postnatally (Mthembu *et al.*, 2021).



Figure(1.5).Risk Factors of Asthma (Ackland *et al.*, 2021)

1.2.6.1. Hereditary

Asthma is linked to more than a hundred genes, while the number is continually expanding. The three primary categories of asthma susceptibility genes include function of the immune system, function of the mucosa, lung function, and progression of disease. (Kulkarni and Kediya, 2022).

Further to environmental factors, asthma susceptibility is significantly influenced by genetics. It was challenging to ascertain the function of asthma-associated polymorphisms and how they translate into disease-relevant pathways because the majority of these genetic changes are located in non-coding genomic regions. Recent developments in genomics and epigenetics, which have started to reveal the molecular mechanisms behind the multifaceted (epi)genetics of asthma, have made the ability to link genetic variants to gene regulatory elements found inside non-coding

regions (Stikker, Hendriks and Stadhouders, 2023).

Even while heredity is a chief hazard factor for developing asthma, environmental and inner exposures, as well as how they interact with genetic variables, also have a substantial impact on the disease's(Kulkarni & Kediya, 2022).

1. 2 . 6. 2. Allergy and Allergen

Pollens, environmental fungi, and house dust mites are examples of common allergens. Bacteria have a dual role in allergy. Usually, they are associated with protection, however, certain bacterial species promote the development and exacerbation of allergic inflammation.(Murrison *et al.*, 2019) .

The most important allergens triggering allergic rhinitis derive from house dust mites (HDM), pollens, pets, and molds. The inhalation of allergens induces a type-2 inflammation in sensitized individuals, leading to the symptoms of allergic rhinitis and asthma with bronchial hyperactivity, inducing inflammation and subsequently tissue remodeling due to persisting allergen exposure (Ankermann & Brehler, 2023).

An allergic response occurs when immune system proteins (antibodies) mistakenly identify a harmless substance, such as tree pollen, as an invader. In an attempt to protect your body from the substance, antibodies bind to the allergen. The chemicals released by your immune system led to allergy signs and symptoms, such as nasal congestion, runny nose, itchy eyes or skin reactions. For some people, this same reaction also affects the lungs and airways, leading to asthma symptoms (Bakiri, Xhetani & Lika, 2021)

Climate change may have specific effects on respiratory health since it

exacerbates the development of respiratory allergies and asthma. Pollen and mold are examples of allergens that can produce pro-inflammatory and immunomodulatory mediators, hastening the development of allergies and IgE-mediated sensitization. Climate change has an impact on the length and intensity of pollen season. Studies show that in response to elevated atmospheric carbon dioxide (CO2) levels, plants increase their capacity for photosynthesis, pollen production, and reproduction. Floods and rainy rains can cause mold development, which in turn can cause severe asthma. The most common methods for assessing the relationship between contaminants in the air and allergic respiratory conditions including sneezing and asthma are pollen and mold allergies. (D'Amato *et al.*, 2020)

1.2.6. 3. Air Pollution

Air pollution poses a significant global health threat, resulting in millions of annual human deaths. The delayed development of respiratory disorders in adults and children that can be linked to prenatal or perinatal exposure to air pollution is a growing concern in the field of human health(Krismanuel & Hairunisa, 2024)

In those with allergic rhinitis, increased sensitivity (allergy) to a substance causes your body's immune cells to release histamines in response to contact with the allergens. Histamines along with other chemicals lead to allergy symptoms. The most common allergens enter the body through the airway (Q. Li *et al.*, 2023)

Air pollution is one of the biggest environmental threats for asthma. (Agache, Canelo-Aybar, *et al.*, 2024)

exposure to air pollution has been linked to an increased risk of asthma development and exacerbation(Agache, Canelo-Aybar, *et al.*, 2024)

previous studies found that short-term exposure to air pollutants was significantly associated with increased the risk of asthma (Zhang *et al.*, 2024)

1.2. 6. 4 Smoking

Passive exposure to environmental tobacco smoke in the home and other places, and active smoking throughout later childhood all have an impact on the respiratory health of children. Parental smoking has been associated with respiratory symptoms and deteriorated lung function in children(Qadri *et al.*, 2024).

The mechanisms described until now include epigenetic modifications with transgenerational impact, altered epithelial barrier with overproduction of epithelial derived cytokines, impaired response to infections, mucus hypersecretion, oxidative stress, amplification of the type 2 (T2), and non-T2 chronic airway inflammation (Agache, Ricci-Cabello, *et al.*, 2024).

It was shown that parents who smoked more frequently had children with breathing disorders or asthma. Parents need to be informed of the harmful effects that tobacco smoke exposure has on their children. Reducing exposure to cigarette smoke in public spaces and workplaces, in particular, is expected to improve respiratory health for the entire population. Implication for public health: Youngsters are more likely than adults to be exposed to passive smoking because they breathe in more toxins per pound of body weight due to their faster breathing rates. Children also take in higher amounts of pollutants from cigarette smoke because they often mouth things. Given that kids spend more time indoors than outside, the home is a major source of exposure (Huang *et al.*, 2023).

1. 2.6. 5 Obesity

Obesity is one of the factors associated with the severity of asthma. Obesity is associated with aggravation of the pathophysiology of asthma, including exacerbations, airway inflammation, decreased pulmonary function, and airway hyperresponsiveness (Tashiro *et al.*, 2024)

In addition to being a significant risk factor and a disease modifier for asthma in both adults and children, obesity is a widespread public health issue. Individuals who are overweight are more likely to develop asthma, and those who already have the disease experience worse quality of life, more symptoms, and more frequent and severe exacerbations of their symptoms. The complicated syndrome of obesity-related asthma encompasses several illness manifestations, some of which are still poorly understood (Peters, Dixon & Forno, 2018)

New evidence from clinical, epidemiological, and experimental studies supports the idea that obesity and asthma are causally related. Metabolic imbalance at the level of fat and sugar metabolites leads to obesity. The occurrence of systemic and local subclinical inflammation reflects the highly active functional nature of adipose tissue. This inflammatory reaction appears to be linked to lung function, airway inflammation, and aggravation of asthma (Miethe *et al.*, 2020)

There are many mechanisms that could explain the relationship between asthma and obesity, and the most obvious is the effect of excess abdominal and thoracic adipose tissue on pulmonary physiology. Obesity may cause alterations in the airways that include increased resistance and breathing work, inefficiency of respiratory muscle, decreased respiratory compliance, or modification in gas exchange (Russjan, 2024).

1.2.6.6 Effect of sex and Age

Asthma is a diverse disease, the age of onset seems to affect the characteristics associated with it. Those patients diagnosed with asthma in adulthood seem to have a weaker response to asthma medication and a worse prognosis than those diagnosed as children(Hisinger-mölkänen, Honkamäki & Kankaanranta, 2022) .

. hildren's baseline characteristics and exacerbation rates varied according to their age group. Clinical guidelines should consider age at time of diagnosis more discretely than the broad range, 5–16 years, as this appears to impact on asthma severity and management (Khalaf *et al.*, 2024).

Men and women suffer the frequency and severity of asthma at various stages in their life due to the illness' heterogeneity. Males are more likely to develop asthma when they are younger (Chowdhury *et al.*, 2021) .

Significant differences may be observed between male and female patients with severe asthma, influencing the asthma pheno-endotyping in both sexes.(Senna *et al.*, 2021).

1.2.6.7.Infections

1.2.6.7.1 Virus

Respiratory virus infections are main triggers of asthma exacerbations(Sverrild *et al.*, 2024).

Viral wheeze is an important risk factor for asthma, which comprises several respiratory types (Havens *et al.*, 2024).

In addition, epidemiological studies show strong associations between asthma and infection with respiratory pathogens, including common respiratory viruses such as rhinoviruses, human respiratory syncytial virus,

adenoviruses, coronaviruses and influenza (Edwards *et al.*, 2012;Y. Li *et al.*, 2023).

Epithelial cells and antigen-presenting cells are two of the many lung tissues that are impacted by viral respiratory infections. The immune system triggers the synthesis of cytokines in the airway epithelial cells, including thymic stromal lymphocytesopoietin (TSLP) and interleukins (IL)-25, IL-33, and IL-33, once it has identified the viral infection. When airborne allergens enter the lungs, these cytokines trigger the TH2 immune response. The TH2 cells' release of certain cytokines, including IL4, IL5, and IL13, leads to an increase in the recruitment of mast cells and eosinophile, which in turn causes bronchoconstriction, cell metaplasia, and airway inflammation(Luz Garcia & del Rosal 2016).

The main causes of illness and airway pathobiology in severe acute exacerbations of bronchial asthma as well as chronic persistent asthma are infectious pathogens. The most common and recurrent dangers in cases of acute exacerbations of bronchial asthma (AEBA), including near-fatal and deadly asthma, seem to be viral agents, including human rhinovirus-C, respiratory syncytial virus, and influenza A. Both viral and, to a lesser extent, bacterial organisms may be involved. Co-infection may also exist and exacerbate the prognosis for hospitalized patients, increasing the risk of critical asthma syndrome in some of them(Sandrock & Norris, 2015).

Dendritic cells (DCs) are one of the key players in antiviral immunity because of their ability to detect pathogens. They can orchestrate an immune response that will, in most cases, lead to viral clearance. Different subsets of DCs are present in the lung and each subset can contribute to antiviral responses through various mechanisms. lung DC-mediated responses to respiratory viruses can lead to the worsening of an existing chronic pulmonary disease such as asthma(De Leeuw & Hammad, 2024)

Viral infections are important triggers of acute wheezing episodes in infancy and the inception and exacerbation of asthma. The 2 most common respiratory viruses to be associated with development and/or exacerbation of asthma, RSV and RV, seem to have disparate mechanisms that lead to disease(Mikhail & Grayson, 2019)

Changes in the immune response to viral infections in genetically predisposed individuals are very likely to be the main factors involved in the association between viral infection and asthma.(Luz Garcia & del Rosal 2016)

1.2.6.7.2 Fungi

Fungi are present in indoor and outdoor environments and have been associated with respiratory disease including childhood and adult asthma. A growing body of evidence from human and animal studies has revealed a link between fungal exposure, especially indoor fungal exposure, with asthma initiation, persistence, and exacerbation(Zhang, Reponen & Hershey).

Fungi have many important roles in paediatric asthma, predominantly by being a source of allergens (severe asthma with fungal sensitization, SAFS), and also directly damaging the epithelial barrier and underlying tissue by releasing proteolytic enzymes (fungal bronchitis).(Bush, 2020).

Several complex biological systems involving lung stroma and immune cells intersect shortly after fungi are inhaled into the lungs. The epithelial lining of the airways senses the presence of fungi and responds by summoning immune cells into action. Upon arrival, a network of diverse immune cells enacts their effective antimicrobial functions. If fungi attach to the epithelial cell surface, penetrate the lung parenchyma, and avoid immune surveillance, then invasive disease ensues. (Wiesner & Klein,

2017).

Patients with asthma and fungal sensitization are more likely to have a higher serum IgE concentration and sensitization to more nonfungal allergens than patients with nonfungal sensitization, suggesting that fungal sensitization may be a marker of an allergic phenotype(Medrek *et al*., 2016).

Few major clinical conditions associated with fungal sensitization and hypersensitive immune response are Allergic bronchopulmonary aspergillosis (ABPA), Allergic fungal rhinosinusitis (AFRS) and Severe asthma with fungal sensitization (SAFS). The most common fungi implicated in these conditions belong to genus *Aspergillus*, although an association with several other fungi has been described.(Singh *et al.,* 2018).

Sensitization to fungi is predominant in men, and it is associated with poor asthma control. In particular, sensitization to Penicillium and Aspergillus is a risk factor for asthma severity(Tanaka *et al.*, 2016).

Atopy is a common feature of severe childhood asthma, and a variety of allergens, including indoor and outdoor fungal contact, can aggravate the condition. Fungal sensitization in adults is linked to greater rates of hospital and critical care unit admissions, as well as raised asthma severity, illness, and death. Fungal exposure in asthma was linked to greater disease severity, elevated bronchial reactivity, greater airway eosinophilophilic inflammation, and more exacerbations in children with chronic symptoms..(Castanhinha *et al.,* 2015).

Patients with pre-existing asthma are frequently discovered to have the growth of mold, which can aggravate asthma symptoms or possibly result in death. Nevertheless, they may also serve as a risk factor for anyone who was previously healthy for getting asthma. Aspergillus, Alternaria,

Penicillium, and Cladosporium are the molds that cause respiratory illnesses most frequently due to allergens; asthmatic patients are particularly susceptible to Aspergillus. Their spores need to be ≤5 μm in size in order to be ingested. In addition to stimulating the innate immune system and Th2 and Th17 pathways, inhaled mold spore germination in the lungs may further raise sensitivity to other mushroom allergens, contaminants, and dust mite allergies.(Jusufovic, 2022).

It has been shown that fungal sensitization increases the risk of having more severe asthma and the risk of dying in asthma patients increases with increased spore exposure. Additionally, fungal sensitivity to Aspergillus and Clasdosporium species increases the risk of adult-onset asthma .(Sandrock and Norris, 2015).

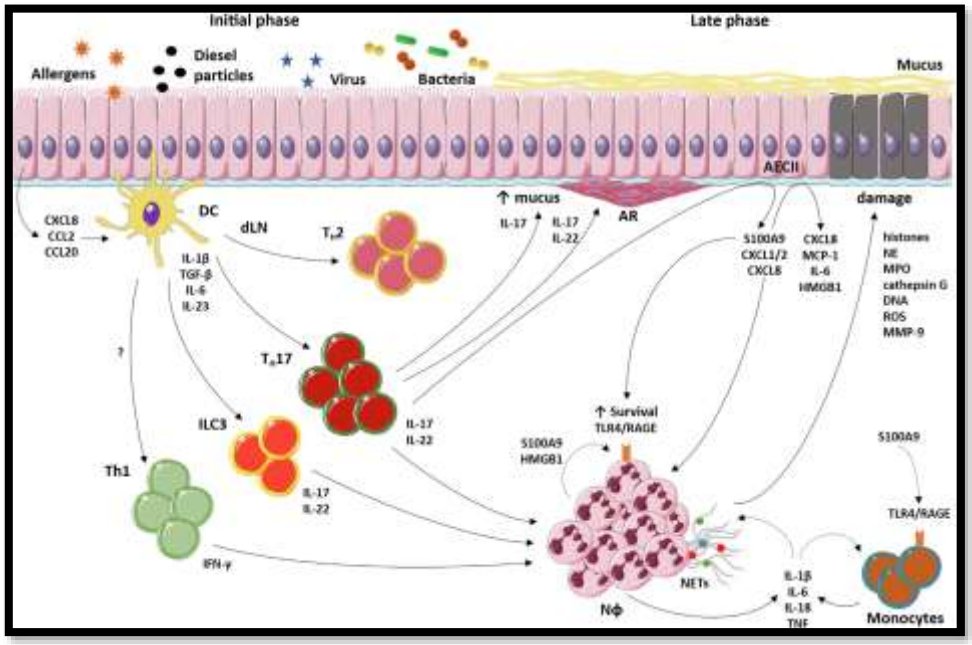
Persistence of fungi in the airways results in sensitization to the Aspergillus species or other mycoses with a T-helper 2 lymphocytesocyte immune response. The effects of immunoglobulin E (IgE) production and peripheral and airway eosinophilophilia are part of the pathological process that may result in bronchiectasis. The typical clinical syndromes of cough, sputum with brown mucus plugs, and wheezing, which are also sometimes associated with the systemic symptoms of fever and weight loss, suggest a diagnosis(Ortega and Izquierdo, 2022).

1.2.6.7.3 bacteria

Bacteria are the most prevalent form of microorganisms. While most bacteria are beneficial to human health, others are pathogenic and can cause mild to severe infections. These bacteria use various mechanisms to evade host immunity and cause diseases in humans. The susceptibility of a host to bacterial infection depends on the effectiveness of the immune system, overall health, and genetic factors. Malnutrition, chronic illnesses,

and age-related vulnerabilities are the additional confounders to disease severity phenotypes (Soni, Sinha & Pandey, 2024).

The complex link between bacterial infections and asthma includes both pathogen-specific immune responses and asthma. In people with allergic asthma, the comorbidities may impact the lung milieu and lead to a preexisting TH2 profile (eosinophilophil inflammation); in more severe cases, they may trigger the immune response to a TH17 profile, which can produce neutrophil or granulocytic inflammation. When some infections cause TH17 cells to proliferate and encourage the entry of neutrophils into the airways, asthma symptoms may deteriorate (Fig 3). If the airway inflammation gets worse, host-directed therapy can be required dependent on the comorbidities and asthma phenotype. (Fraga-Silva *et al.*, 2023).



Figure(1.6). Exacerbation of asthma and bacterial infections.

Among the respiratory tract cultures, the frequency of Gram-negative bacterial strains was higher than Gram-positive bacterial strains. *Pseudomonas aeruginosa* was the dominant pathogen in both the adult respiratory ward (*n* = 156, 21.49%) and RICU (*n* = 975, 35.67%), whereas *Staphylococcus aureus* (*n* = 66,

19.19%) was the most common bacterium in the pediatric ward(Duan *et al.*, 2020).

Gram-positive organisms, including the pathogens *Staphylococcus aureus, Streptococcus pneumoniae ,* have dynamic cell envelopes that mediate interactions with the environment and serve as the first line of defense against toxic molecules. Major components of the cell envelope include peptidoglycan (PG), which is a well-established target for antibiotics, teichoic acids (TAs), capsular polysaccharides (CPS), surface proteins, and phospholipids. These components can undergo modification to promote pathogenesis(Rajagopal & Walker, 2017).

several groups of staphylococcal proteins are able to interact with the human airway mucosa and its immune system, including the epithelium, ILCs, dendritic cells, T and B cells, mast cells, basophils and eosinophile. Some of these interactions are shared by other bacteria or infectious agents, such as the release of IL-33 from the epithelium; others are specific to the superantigenic properties of the S. aureus proteome(Bachert *et al.*, 2020).

Infection with *S. pneumoniaee* was related to adult asthma exacerbation(Vroman, van den Blink & Kool, 2015).

Acute asthma attacks in children have been linked to gram-negative bacteria in the lower respiratory tract. These microorganisms and related cytokines may contribute to children's asthma getting worse. (Kim *et al.*, 2021).

Gram-negative organisms comprise a large portion of the pathogens responsible for lower respiratory tract infections, especially those that are nosocomially acquired, and the rate of antibiotic resistance among these organisms continues to rise(Wenzler *et al.*, 2016) .

Acute exacerbation of asthma was associated with infection in most

patients. Gram-negative bacteria and *S. pneumoniaee* form a relevant part of the microbial pattern of exacerbation of asthma(Ahmed, Embarek Mohamed & Hassan, 2017).

Effective preventive and therapeutic intervention options are now hindered by the lack of a comprehensive understanding of the underlying mechanism of the viral-bacterial synergy that drives illness progression. In addition to the harm that viruses inflict on the epithelial cells that line the airways, which allows bacteria to proliferate and become more invasive in the lower respiratory tract, compromised immune function following a viral infection has also been connected to a higher chance of developing secondary bacterial infections(Sumitomo & Kawabata, 2024).

A major factor in the pathophysiology of lower respiratory tract infections (LRTIs) is bacteria that colonize young children's upper respiratory tracts (URTs). Evidence linking *H. influenzae or Klebsiella* spp. to lower respiratory tract infections (LRTIs) was found in the URT, although the relationship with *S. pneumoniaee* was less clear-cut.(Claassen-Weitz *et al.*, 2021) .

1.2.6.7.4 Normal flora

The lung microbiome in adult asthma is the product of a long period of dynamic shaping by microorganisms and the host from infancy onwards(White & Huang, 2022).

The microbiome of healthy lungs is mainly derived from the upper respiratory tract (URT) microbiome but also has its own characteristic flora. The selection mechanisms in the lung, including clearance by coughing, pulmonary macrophages, the oscillation of respiratory cilia, and bacterial inhibition by alveolar surfactant, keep the microbiome transient and mobile, which is different from the microbiome in other organs(Li, &

Zhou, 2024).

Characteristics of airway microbiota might influence asthma status or asthma phenotype(Li *et al.*, 2023).

Patients with asthma have an altered airway microbiota, with specific bacteria associated with severe asthma and the eosinophilophilic inflammatory phenotype(Li *et al.*, 2017).

A growing body of research has demonstrated that early exposure to a variety of microbes may modify the host immune response and affect an individual's susceptibility to atopic disease. The condition known as asthma is complex and diverse. It has been demonstrated that lower airway dysbiosis plays a role in the pathophysiology of asthma, particularly neutrophilic asthma. One theory is that exposure to bacterial lipopolysaccharide (LPS) can cause an increased Th-17 response when a certain type of bacterium is prevalent(Zhang *et al.*, 2020).

The host's immune system undergoes molecular changes due to bacteria and their metabolites; these changes can impact cell differentiation, cell responses, and the release of proinflammatory or protective molecules such antibodies, cytokines, and interleukins. Because microbial dysbiosis promotes opportunistic pathogen invasion brought on by unbalanced immune responses, it can create changes in the inflammatory system and contribute to the development of allergy disorders, particularly asthma(Green *et al.*, 2022).

Dysbiosis, defined as deviation from a normal microbial composition, is associated with a number of adverse biological phenomena, sometimes with clinical consequences. In the lung, dysbiosis can have a significant impact on the development and progression of respiratory diseases, emphasizing the clinical need to understand the biology of thelung

microbiome(Natalini, Singh & Segal, 2023).

Multiple mechanisms have now been described, through which bacteria can induce regulatory responses or dampen inflammatory processes. Both bacterial cell wall components and metabolites from the microbiome have been associated with immunoregulatory effects within the mucosa(Sokolowska *et al.*, 2018).

The host immune system is able to recognize microbial exposures on any mucosal surface in the human body. Actually, the microbial products seen in the lower airways are generated by the equilibrium between microbial immigration and microbial clearance7. However, in contrast to other mucosae, the lungs' main physiological role is to enable gas exchange, which needs a lower bacterial burden. With respect to other mucosae, the lower respiratory tract functions differently in terms of oxygen tension, protein composition, surfactant presence, and other environmental factors. To maintain a low bacterial load, there is also an important reliance on processes that help eliminate microorganisms that enter the lower airways. Mechanical processes like coughing and mucociliary clearance are among them(Natalini, Singh & Segal, 2023).

patients with asthma have an increased prevalence of *Streptococcus pneumoniaee* carriage during birth and childhood(Lee and Park, 2023).

Sputum microbiota in severe asthma differs from healthy controls and non-severe asthmatics, and is characterized by the presence of *Streptococcus spp* with eosinophilophilia. Whether these organisms are causative for the pathophysiology of asthma remains to be determined(Murrison *et al.*, 2019)

1.2.7. Diagnosis of Asthma

In practice,diagnosis of asthma should be established by considering

characteristic symptoms patterns plus other test dignostic.Asthma is distinguished by fluctuating symptoms, which may include wheezing, dyspnea, chest tightness, and cough. It is also characterized by variable limitation in expiratory airflow(Furukawa *et al.*, 2024) .

1.2.7.1 Pulmonary function tests (PFT)

Pulmonary function tests provide quantitative assessment of physiological properties in respiratory system including the lungs and chest wall. Spirometry is the first step as a screening and diagnostic test to investigate the existence and severity of obstructive or restrictive ventilatory disorders(Alamri, 2021).

Spirometry is the standard pulmonary function test for assessment of lung function as well as severity of asthma among all age groups. Forced expiratory volume in 1 s (FEV1) is the most widely used spirometry parameter which has been shown to have best sensitivity and correlation with severity and control of asthma in various studies. Global Initiative for Asthma (GINA) guidelines have also recommended usage of FEV1 in spirometry for diagnosis and monitoring of lung function in asthma(Gupta, 2023).

To confirm the diagnosis if an airway obstruction is found reversible based on an FEV1 (Forced expiratory Volume in 1 second) increase of >12 % and >200 ml after administering 200–400 μg salbutamol(Horak *et al.*, 2016).

1. 2.7.2. Fractional exhaled nitric oxide (FeNO) in Asthma

Nitric oxide (NO) is increased in the airways and serum of patients with allergic asthma(Chacon *et al.*, 2024)

Several studies have shown that measuring eNO levels can help guide asthma management in pediatric patients. For example, a study published

in the Journal of Pediatrics found that monitoring eNO levels in children with asthma helped reduce the need for oral corticosteroids and emergency department visits. Another study published in the European Respiratory Journal showed that adding eNO measurement to standard clinical care improved asthma control in children(Hassan *et al.*, 2024)

Fractional exhaled nitric oxide (FeNO) is a marker of eosinophilophilic inflammation and tailoring asthma medications in accordance to airway eosinophilophilic levels may improve asthma outcomes such as indices of control or reduce exacerbations, or both.(Petsky *et al.*, 2015)

2. 2.7.3. The Role of Immunoglobulin E (IgE) in Asthma

Immunoglobulin E (IgE)- mediated allergy is the most common hypersensitivity dis-ease affecting more than 30% of the population. Exposure to even minute quantities of allergens can lead to the production of IgE antibodies in atopic individuals(Shamji *et al.*, 2021)

A major factor in allergic asthma is IgE. IgE-specific receptors on mast cells and basophils bind to antigens such as pollens and house dust mites, which is why allergy sufferers' IgE antibodies are specific for these substances. The binding of IgE molecules triggers the production of cytokines (IL-4, TNF-α, and IL-5) as well as intermediates (arachidonic acid metabolites and histamine) that are critical for both early- and late-stage allergic reactions and the subsequent entry of eosinophile into the respiratory tract.(Mubarak, Shakoor and Masood, 2019).

IgE antibodies specific for bacterial antigens are found in the sera of allergic individuals. This implies that some bacterial factors are allergens, eliciting a specific type 2 immune response. However, to date, only a few of these are molecularly defined(Murrison *et al.*, 2019)

wherein serum total IgE, ECP, and IFN-γ may have an important role on acute exacerbation of asthma(Kama *et al.*, 2022)

Asthma guidelines recommend identifying causal or trigger allergens with specific IgE (sIgE) testing after a diagnosis of asthma has been made. Blood tests for total and sIgE are accessible and yield quantifiable results for tested allergens, useful for detecting sensitization(Demoly *et al.*, 2022)

Both abnormal sputum eosinophilophilia and total serum IgE levels are correlated with the severity of asthma(Issa *et al.*, 2020)

1. 2. 7.4 Role of Eosinophilophil and neutrophil in asthma

Eosinophile represent 1–4% of circulating leukocytes and have a pivotal role in host defence against helminths, the propagation of allergic conditions, and immune and inflammatory networks. They possess receptors for many inflammatory mediators and produce and release an array of biologically active molecules, including cytotoxic proteins, lipid mediators, chemokines, and cytokines(Agnello *et al.*, 2021)

Neutrophils represent the most prevalent leukocytes and the most abundant innate cell population in systemic circulation, making up about 40% to 70% of the total leukocyte count(Riley and Rupert, 2015)

neutrophilic inflammatory phenotype in asthma is associated with increased bacterial burden in airways, along with a disordered airway microbiome and excessive airway inflammation. Airway dysbiosis is one of the key factors underlying the heterogeneity of asthma inflammatory phenotypes. It promotes an understandin of the relationship between airway neutrophilia and bacterial microbiota and may help in the treatment and management of asthma(Yang *et al.*, 2018)

Eosinophile are specialized leukocytes, primarily found in tissues, and in

the respiratory system, airway mucosa, and airways(Popović-Grle *et al.*, 2021)

Eosinophile are likely to contribute to the development of asthma exacerbation. This process can involve cytokines, such as IL-5 or GM-CSF, chemokines, such as CCR3 ligands, matricellular proteins, a danger signal, and other cells, such as neutrophils or mast cells(Nakagome & Nagata, 2018).

Blood eosinophilophil count and gravel other variables routinely available in-patient records may be used to predict frequent asthma exacerbations(Czira *et al.*, 2022)

The neutrophil-tolymphocytesocyte ratio, a blood count-derived parameter, is a marker of systemic inflammation, combining innate and allergic inflammatio markers(Arwas *et al.*, 2023)

There are many parameters to assess asthma phenotype including complete blood count, specifically the neutrophil and eosinophilophil count. Blood eosinophilophil and neutrophil counts had been evaluated with asthma control and exacerbations in adults, but literature is limited in children(Agarwal & Jat, 2023)

Airway colonisation with potentially pathogenic micro-organisms in asthma is associated with more severe airways obstruction and neutrophilic airway inflammation(Campbell, Gleeson & Sulaiman, 2023)

In addition to blood eosinophile, the blood neutrophil count reflects underlying inflammatory patterns and indicates important differences in asthma clinical features and outcomes(Flinkman *et al.*, 2023)

The defining characteristics of asthma heterogeneity include eosinophilophilic, noneosinophilophilic, or mixed granulocytic

inflammations. Individuals suffering from asthma may produce either a TH2-prone or TH17-prone immunological response, contingent upon the priming of lung immune and structural cells.(Fraga-Silva *et al.*, 2023)

1.2.7.5 Role of lymphocytesocyte

Lymphocyte are key components of the adaptive immune response. They make up approximately 20–40% of the total leukocyte count(Agnello *et al.*, 2021)

T cells also generate enormous cytokines and chemokines to amplify the immune response, thus enhancing airway smooth muscle contraction, mucus secretion, and airway hyperresponsiveness (AHR), as well as T cell proliferation in asthma (Zhu *et al.*, 2020)

lymphocytesoid cells able to produce the classical type 2 cytokines. The role of Th2 cells and IL-4 is crucial in the pathogenesis of allergic as supported by asthma models. IL-13, shares many biological functions with IL-4 such as induction of IgE synthesis and regulation of eosinophilophil trafficking(Matucci *et al.*, 2021)

Neutrophils are known to play a role in airway inflammation and are activated in inflammatory lung diseases such as asthma. In adult studies the neutrophil/lymphocytesocyte ratio (NLR) was found to be a possible biomarker for both airway and systemic inflammation(Nacaroglu *et al.*, 2016)

neutrophil-to-lymphocytesocyte ratio is a promising marker to distinguish children with exacerbated asthma from healthy children(Zhu *et al.*, 2021)

1. 2. 8. Treatment of Asthma

Asthmatic medication is based on a progressive and control-based method that comprises an iterative cycle of evaluation, modification of the

medication and review of the response designed to decrease symptom burden and risk of exacerbations. Treatment for inflammation is the cornerstone of managing asthma.Asthma control medications reduce airway inflammation and help to prevent asthma symptoms; among these, inhale corticosteroids (ICS) are the mainstay in thetreatment of asthma, whereas quick-relief (reliever) or rescue medicines quickly ease symptoms that may arise acutely. Among these, short-acting beta-agonist(SABAs) rapidly reduce airway bronchoconstriction (causing relaxation of airway smooth muscles;Papi *et al.*, 2020)

Inhaled corticosteroids (ICS) remain the mainstay of asthma treatment, along with bronchodilators serving as control agents in combination with ICS or other reliever therapy(Calzetta *et al.*, 2022)

In the future, a fast and easy method for the detection of pathogens is required for early treatment of viral or bacterial infections in asthma exacerbation inpatients(Iikura *et al.*, 2015)

Antibiotic exposure is associated with changes in immunological markers and the biological environment of the airways, which can result in allergies like asthma. (Alamri, 2021).

The asthma severity level is defined according to the response to treatment: Mild asthma well controlled by step 1 or 2 therapy and Moderate asthma Asthma well controlled by step 3 or 4 therapy and Severe asthma Asthma Partially controlledcontrolled by maximum inhaled therapy, or asthma control lost when this therapy is reduced; step 5 therapy required(Lommatzsch, Buhl & Korn, 2020)

Chapter Two

Materials and Method

2.1. Subjects and study setting:

The study includes (100) participants previously diagnosed as acases of moderate –sever asthma by physicians. According to the patient's ability, sputum or cough swab samples were collected from each patient for bacterial culture. Also, 3 ml of whole blood was collected (2) ml in an EDTA tube to count the number of neutrophils, eosinophile, and lymphocyte, and (1) ml of blood was placed in a gel tube to measure the total serum IgE. Samples were taken from both sexes (69) males and (31) females, aged between (5-16) years, at the karbala hospital for teaching children, And AI- Imam AL-Hussein Teaching Medical City. The data were collected from October \2023\ to May (2024). Case information sheets data . were implemented for each patient in with a detailed questionnaire.

2.1.1 Inclusion criteria:

Diagnosed cases of moderate –sever asthma ,aging 5-16 years.

2.1.2 Exclusion criteria:

Patients used antibiotics in the last 2 weeks .

2.1.3 Ethical approval:

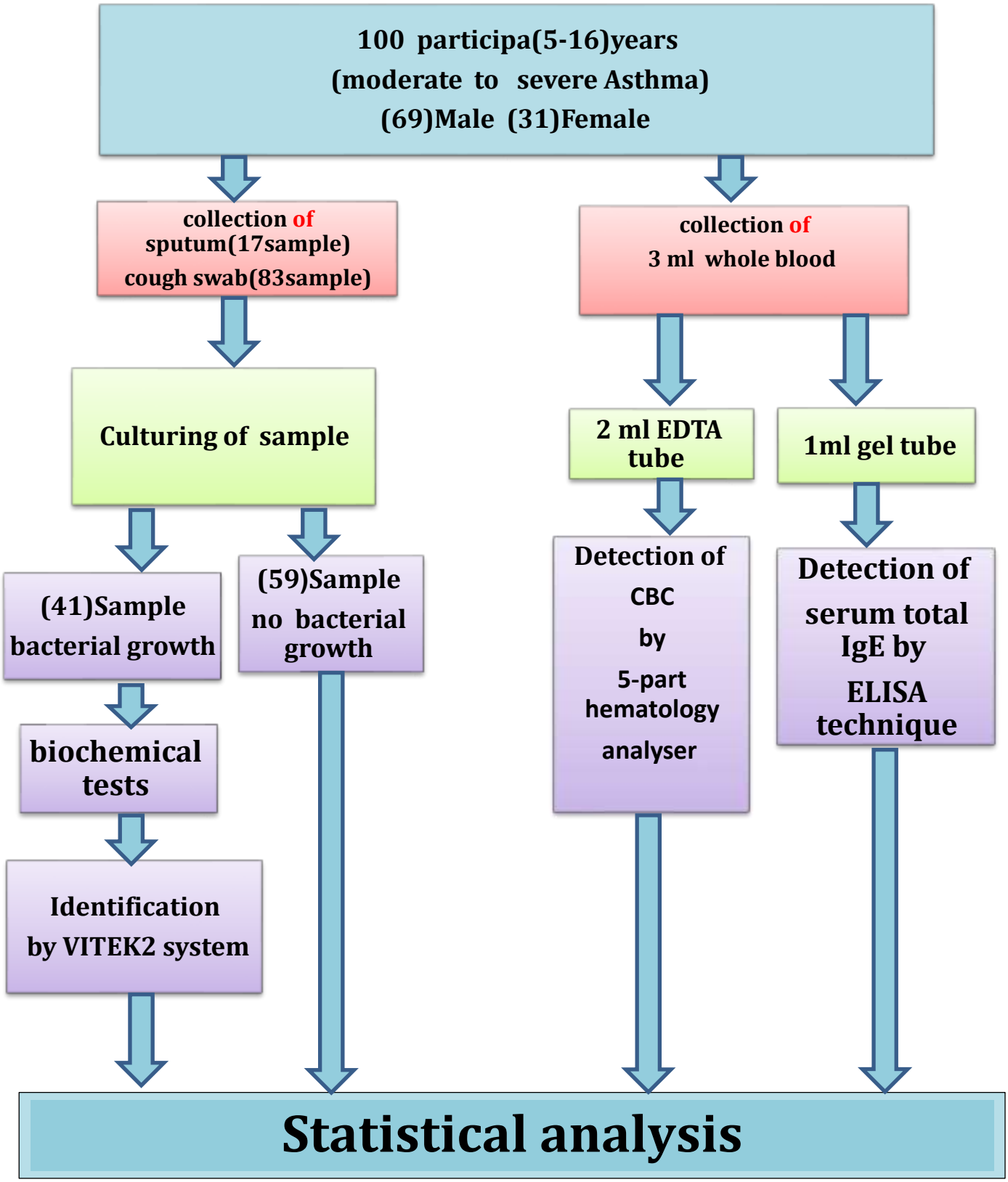
Ethical approval was obtained from Karbala Health Directorate. In addition, verbal permission was taken from the patients and /or their parents before taking the samples. Health measures and safety measures were taken during sampling.names were removed and replacedy identification codes and data used exclusively for research pur poses

2.1.4 Study Design:

This is an observational prospective cross-sectional study

2.1.4.1 Study Design Scheme

The design of study was illustrated in Figure(2.1).



2.2. Materials:

2.2.1 Instruments used in the study :

Instruments and equipments are indexed in table 2.2 & 2.3

Table (2.1): Instruments used in the study

Equipment and instruments manufacturing	manufacturing company	Country of origin
Autoclave	Hirayama	Japan
Biological safety cabinet	EuroCloneSafemate	Italy
Burner	Amal	Turkey
Centrifuge	Hettick	Germany
Cotton	Dunya	Iraq
Cotton	Dunya	Iraq
Cup collection of sputum (sterile)	daltalab	Italy
Cup collection of sputum (sterile)	daltalab	Italy
Deep freezer	L G	Korea
DensiCHEK	Bio merieux	France
drying rack.	. ALS	. China
ELIZA Devices (washer & reader)	Human	China
ELIZA printer	Epson	Japan
Filter Paper	Satorius membrane filter	Germany
flasks (different size)	Jlassco	India
forceps	Himedia	India
gloves.	Al Rawan	China

(gel tube)&(plain tube)	Al Rawan	China
(gel tube)&(plain tube)	Al Rawan	China
Incubator	Memmert	Germany
Light microscope	Olympus	Japan
Loop	Himedia	India
Multichannel micropipette set	SLAMED	Germany
Petri dish	Dolphin	Syria
pipette tips.	Biozek	Netherlands
Refrigerator	LG	Korea
Slides	Himedia	India
Swab media	Himedia	India
Syringes 5 ml	Al Rawan	China
Syringes 5 ml	Al Rawan	China
Timer watch	Dragon	China
VITEK 2 compact system	Bio merieux	France
vortex	Clay Adams	Germany
Water bath	Memmert.	Germany
water distillatrory	GFL	Germany
5-part Hematology Analyser	Swelab	Sweden

2.2.2. Chemicals and Biological materials :

The chemicals and biological materials are listed in table (2.4).

Table (2.2): Chemicals and biological materials which are used in the study.

Chemicals and biological materials	Company	Country of origin
Gram's stain kit	Biolife	Italy
Oil immersion	BDH	Englan
Hydrogen peroxide (H2O2)	SDA	Iraq

2.2.3. Culture media :

The culture media used in the present study are in table (2-4).

Table (2.3): Culture media used in the current study.

Culture media	Company	Country of origin
Blood agar base (BAB)	Himedia	India
MacConky agar	Oxoid	England
Manitol salt agar	Oxoid	England

2.2.4.Commercial kits:

The commercial kits used in the present study are in table (2.5).

Table (2.4): The Commercial kits which are used in the study

Kits	Company
Total IgE ELISA Kit	demeditic
ID (VITEK2) cards cassette	BioMerieux
5-part Hematology Analyser	Swelab

Table (2.5): shows the Reagents of Human total IgE ELISA Kits

Components	Quantity
Pre-coated ELISA Plate	12*8 weel strips x1
Standard Diluent **0, 5, 25, 100, 250, 1000 IU\ ml**	1x1ml 5x200µl
Monoclonal anti- LgE coated microtiter strips	12 ml
Stop Solution	12 ml
Substrate Solution	12 ml
Enzyme congojugate(goat anti- IgE- HPR	22ml
Wash Buffer Concentrate	60 ml
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pics

2.3. Method:

2.3.1.1 Specimen Collection

The priority for taking samples was to take sputum from the patient, and if he was unable to give sputum, a cough swab was taken, and then the samples were taken directly to the laboratory. And the specimens were cultured on blood agar and macconkey agar and mmanitol salt agar , after culturing can incubated for 18- 24 hour in 37^C in the laboratories of Imam

Hassan Al-Mujtaba Teaching Hospital .

2.3.1.1.1 sputum Collection

sputum was collected from a patient with exacerbated asthma , at the morning in a sterile container after rinsing of the mouth of a patient with saline or water before expectoration and the patient then asked to provide a deep-coughed specimen to put the sputum in a sterile container with attempts to minimize contamination by saliva, then the specimens were transported immediately to the laboratory

2.3.1.1.2 cough swab (oropharyngeal swab) (OP) Collection

Many children, especially young children, cannot produce a sputum sample. For these children a cough swab was used(**OP**). It is a swab placed on a stick that is placed as far into the back of the throat as possible immediately after coughing. For children who cannot cough on demand, rub the swab on the back of the throat, ideally immediately after the child coughs.. The posterior pharyngeal wall was scanned OP swab: After gently opening the mouth (with the aid of a tongue depressor in uncooperative children), a cotton-tipped swab was passed to sample the posterior pharynx or induce a cough. . and the immediately sent to the microbiology laboratory(Zampoli *et al.*, 2016); (Fenn *et al.*, 2022)

2.3.1.1.3. Blood sample.

Approximately 3 ml of venous blood was drawn from each participant which was obtained by disinfecting the anticup puncture with 70% ethanol and then making a venous puncture via disposal syringes after applying a tourniquet. 2 ml of blood was dispensed into an EDTA tube (to perform a five-differentiated blood cell count). Also, 1 ml of blood was dispensed into a gel tube and left to clot, then the serum was separated by

centrifugation at 3000 rpm for 15 minutes. The serum was then transferred to a new striated tube and stored in deep freeze (−20°C) for use in immunoassays. (total IgE)

2.4. Laboratory methods

2.4.1. Preparation the Media:

Preparation of Culture Media: A set of culture media was prepared following the company's instructions and sterilised using autoclaving(Hirayama) at a temperature of 121oC for a duration of 15 minutes.. After that, culture media incubated for (24) hours to avoid the contamination, and then it was stored at (4˚C) until use. (MacFadden, 2000).

2.4.1.1 . culturing Media (Streak method) :

Used for the isolation of bacteria in pure culture from clinical specimens. One swab full of the specimen is transferred onto the surface of a well dried plate. Spread over a small area at the periphery. The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate. On incubation, separated colonies are obtained over the last series of streaks. (MacFadden, 2000).

2.4.1.2 types of media used in method of culturing

2.4.1.2.1 : Blood Agar

As directed by Liofilchem/Italy, the manufacturer, prepare this culture medium by dissolving 40.0 gm of blood agar base in 1000 ml of distiller water. After heating the medium to a temperature of full dissolution, it was autoclaved for 20 minutes at 121 °C, cooled to 45 °C, and 5% fresh human blood was added. It was employed to assess the

bacterial isolates' capacity to hemolyze red blood cells and as an enrichment medium. (MacFadden, 2000).

2.4.1.2.2 : MacConkey Agar

It had been prepared by dissolving (50)gm of the medium in (1000ml) of distilled water, heated for boiling, sterilizing in an autoclave and used to detect the growth of gram-negative bacteria (MacFaddin, 2000). Differential and selective media;

2.4.1.2.3 Mannitol Salt Agar

111 grams of powder are dissolved in one liter of distiller water, and the mixture is then sterilized in an autoclave for 20 minutes at 121 degrees Celsius, depending on the manufacturer. The culture media was added to sterilized Petri dishes at a temperature of 45°C. This kind of medium ferments mannitol and is specific for isolating staphylococci. (MacFadden, 2000).

2.4.1.2.4 Nutrient Agar

Depending on the manufacturer, it is made by dissolving 28.0 grams of powder in one liter of distiller water, followed by 20 minutes of autoclaving at 121 degrees Celsius to sterilize it. When necessary, it has been utilized to culture, activate, and isolate microorganisms. (MacFaddin, 2000).

2.4.1.3 Preparation of solutions and indicators

Gram stain and regular saline were utilized as ready-made reagents and solutions, and the following in-stand reagents were created as needed:

2.4.1.3.1 Oxidase reagent.

The manufacturer's firm states that 0.1 gm of tetramethyl-para-

phenylenediamino dihydrochloride was dissolved in 10 ml of distilled water to make the oxidase reagent at a concentration of 1%. Finding microorganisms that might produce the cytochrome oxidase enzyme can be done with the use of the oxidase test. The test separates the Enterobacteriacea and Pseudomonacea oxidase-positive and oxidase-negative groupings. (Green, and Goldman, 2021).

2.4.1.3.2 Catalase reagent.

The catalase reagent was made at a concentration of 3%, according to the manufacturer's company, by mixing 1 milliliter of 30% hydrogen peroxide with 9 milliliters of pure water. It has been employed to identify bacteria that are capable of producing the catalase enzyme. (Green, and Goldman, 2021).

2.5 Specimens culturing

Swabs were streaked on blood and MacConkey agar and incubated for 24 hours at 37°C in an aerobic environment. The next day, the plates were checked for growth, and a pure colony of every variety of bacterium was subsequently made. The automated VITEK2 system's specialized cards, morphological, biochemical, and microscopic features were used to identify the isolated bacteria. (Dubey and Maheshwari, 2023).

2.6 . Gram stain

2.6.1 . Preparation of smear.

The smear on a glass slide was covered with few drops of one of the primary stains (crystal violet). The primary stain rendered all the bacteria uniformly violet. After a minute of exposure to the staining solution, the slide was washed in water, treated with a few drops of Gram's Iodine and allowed to act for a minute and again washed in water and then decolorized

in absolute ethyl alcohol or acetone. The process of decolorization was fairly quick and should not exceed 30 seconds for thin smears. After the smear was decolorized, it washed in water without any delay. The smear was finally treated with few drops of counterstain such as dilute carbol fuchsin, neutrophiltral red or safranin. finally washed in water; excess water was removed using a blotting paper, dried in air and heat fixed before observed under the microscope (Nain *et al.*, 2018).

2.7 Sterilization Methods .

The culture medium used in this investigation were autoclaved at 121°C for 15 minutes to ensure sterilization. B. Glassware is sterilized using dry heat for two hours at 180 degrees Celsius in an electric oven.

2.8 Bacterial Profile identification

2.8.1 . Morphological Tests

Tests were conducted on several aspects of the colonies, including their size, color, texture, boundaries, and form..

2.8.2 .Microscopic Characteristics

Following Gram stain staining, bacteria were seen under a light microscope. A tiny portion of a bacterium colony was applied on a clean slide using a drop of regular saline, exposed to flames to fix it, then covered in crystal violet, Iodine, alcohol, and safranine to counterstain before being studied under an oil immersion lamp.

2.9 . Biochemical test

2.9.1 Catalase test

A bacterial colony was combined with a few drops of 3% H2O2 on a slide for the test, and after 10 seconds, bubbles were supposed to develop.

The catalase enzyme produced by these bacteria will neutrophiltralize the hydrogen peroxide, causing bubbles to develop and a positive test result. (Shoaib *et al.*, 2020)

2.9.2 Oxidase test

To conduct the test, 1% tetramethyl-p-phenylenediamine dihydrochloride, a synthetic electron donor, was impregnated onto filter paper, and the paper was then dried. After 10 seconds, the color shift of the bacterial colonies is seen on a paper strip. (Shoaib *et al.*, 2020)

2.9.3 Coagulase test (tube method)

Bacteria are put to plasma in a test tube to conduct the tube test. A favorable response is indicated by plasma coagulation (including any thickening or development of fibrin threads) within 24 hours. Testing for clotting (without shaking) in plasma usually takes place after 4 hours, as early coagulation may occur and the plasma may revert to liquid within 24 hours. (Shoaib *et al.*, 2020)

2.10. Identification by using automated methods [VITEK2] system:

Automated methods are the most accurate and quickest for identifying bacteria. The components of the VITEK2 system consist of plastic reagent cards containing microliter amounts of different biochemical test medium in 30 wells to create a biochemical profile that is used for organism diagnosis.(Maina and Kagotho, 2014).

2.11. Maintenance of bacterial isolates:

The bacterial isolates' maintenance was done as the following

2.11.1. Short-term storage

For several months, the unadulterated bacterial isolates were preserved

in screw-capped universal tubes filled with brain heart infusion agar slant and cultured for twenty-four hours at 37ºC. After being securely covered in Parafilm, the slants were stored at 4ºC for three months. (Benson, 2002.)

2.11.2. Long-term storage

After 18 hours, a loop of overnight pure bacterial culture was used to inoculate a brain heart infusion broth, which was then incubated at 37°C. For two to eight months, the inoculate was kept at -20°C with glycerol added at the final concentration of 20%. (Ali, 2023)

2.12. immunological markers

2.12.1. Determination of the level of total immunoglobulin e(total IGE)

2.12.1.1. Principle of sandwich elisa technique:

The foundation of the complete IgE elisa is the enzyme immunoassay (eia) concept. The microtiter strips have a monoclonal mouse-anti-human ige antibody attached to their surface. The antihuman-ige-peroxidase conjugate and undiluted patient serum or ready-to-use standards are pipetted into the microtiter plate wells. The two antibodies and the serum ige form a sandwich complex. To get rid of unbound material, the plate is washed with diluted wash solution after being incubated for 30 minutes at room temperature. After pipetting the substrate solution, the wells are incubated for 15 minutes to cause the production of a blue dye. The addition of a stop solution, which turns the color from blue to yellow, ends the color development process.

2.12.1.2. Preparation of reagents

Washing solution: diluted before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°c for 15 minutes.

- all other reagents ready to use , must be brought to room temperature before use, but should not be left at this temperature longer than necessary.

- microtiter strips 12 strips with 8 breakable wells each, coated with mouse monocle nalanti-IgE. Ready-to-use

- . Calibrators (standards) 1 ml (0 iu/ml), 5x 200 µl (5, 25, 100, 250, 1000 iu/ml), human serum diluted with pbs. Calibrated against the 2nd international standard 75/502. Addition of 0.1% sodium azide. Ready-to-use.

- . Enzyme conjugate 22 ml, goat anti-human-ige-hrp, in protein-containing buffer solution. Ready-to-use.

- substrate 12 ml, tmb (tetramethylbenzidin). Ready-to-use.

- stop solution 12 ml, 1 n acidic solution. Ready-to-use.

2.12.1.3. Assay procedure

1. Preparing a sufficient amount of microtiter wells for the standards and samples in duplicate as well as for a substrate Blank.
2. Pipetting 10 µl each of the undiluted samples and the ready-to-use standards together with 200 µl of conjugate into the wells. Leave one well empty for the substrate blank.

3. Covering the plate with the re-usable plate cover and incubate at room temperature for 30 minutes.

4. Emptying the wells of the plate (dump or aspirate) and add 300 µl of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

5. Pipetting 100 µl each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted

6. Covering the plate with the re-usable plate cover and incubate at room temperature for 15 minutes in the dark (e.g. Drawer).

7. To terminate the substrate reaction, pipetting 100 µl each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

8. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

2.12.1.4.. Calculation of result.

Quantification the mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%. The ready to use calibrators of the total ige elisa are defined and expressed in international units (iu), The absorptions of the standards are graphically plotted versus their concentrations for a quantitative assessment. The concentration values for each patient sample may then be retrieved in respect to their absorptions from the resultant reference curve. As an alternative, using an electronic gadget is an option. The findings can also be computed using standard computerized data processing algorithms, such as logit-log, spline, and four parameters. Any sample that reads higher than the highest standard needs to be properly diluted using zero standard before being retested. The dilution factor needs to be multiplied by the result. Every experiment done in the lab should begin with the establishment of the standard curve.

2.13. Statistical analysis

The data analysis for present investigation was generated using The Statistical Package for the Social Sciences software, version 26 (IBM, SPSS, Chicago, Illinois, USA). Descriptive statistics was performed on the

patient's data of each group. Data was analyzed for means, and the standard deviation was computed for the continuous variables, whereas frequency was used for computing the qualitative data. The mean of the investigated biomarkers were compared between the studied groups using t-Test; Chi-Square analysis was employed to significant compare between percentages; Differences among groups were analyzed using one-way ANOVA analysis of variance. Also, Pearson correlation coefficients were calculated to check the relationship between the studied markers. All hypothesis test results with two-sided p-values less than 0.05 were deemed statistically significant.. (Basher, 2003)

Chapter Three

Results

3. The results

3.1 Demographic data of Asthmatic patients

Table (3-1) shows the demographic data of patients, that including distribution of patients according to age, BMI, residency, sex, family history,symptoms affection by exercise, and presence or absence of animals in house .regarding age the Patients were divided into three age categories: 5-8 y, 9-12 y, and 13-16 y; the results of statistical analysis revealed that majority (57%) of asthma patients within the age category 5-8y, with statistical significant differences (p=0.0001). According to BMI values, patients were dividing into four BMI categories: Underweight, Normal, Overweight, and Obesity; a significant difference was reported (p=0.0001), where the higher percent (56%) of patients were found within the Normal category. The distribution of patients according to their Residency showed no significant differences (p=0.2301) between urban (44%) and rural groups (56%). Also, significant (p=0.0001) differences were found in patients groups according to sex, the majority of patients were male (69%) versus to (31%) female. regarding to family history, patients were divided into two groups (Negative family (FH^-), and postive family (FH^+)), where (65%) of asthma patients found in FH+ group, with significantly difference (p=0.0027). Asthma patients also dividing according to syptoms affection by exercise into two groups: affected (73%) and non-affected (27%), the results of statistical analysis indicated a significant difference (p=0.0001) between these groups. Furthermore, the patients dividing according to presence or absence the animals in house, however, the results did not show any significant differences between these groups, (p=0.1615).

Table (3-1): Demographic data for patients

Age group (year)					
5-8 yr	**9-12 yr**	**13-16 yr**		**Total**	***P value* (P ≤ 0.05)**
57 (57%)	27 (27%)	16 (16%)		100 (100%)	0.0001*
BMI					
Underweight	**Normal**	**Overweight**	**Obesity**	**Total**	***P value* (P ≤ 0.05)**
22 (22%)	56 (56%)	13 (13%)	9 (9%)	100 (100%)	0.0001*
Residence					
Urban	**Rural**	**Total**		***P value* (P ≤ 0.05)**	
44 (44%)	56 (56%)	100 (100%)		0.2301NS	
sex					
Male	**Female**	**Total**		***P value* (P ≤ 0.05)**	
69 (69 %)	31 (31%)	100 (100%)		0.0001*	
Family history					
FH-	**FH+**	**Total**		***P value* (P ≤ 0.05)**	
35 (35%)	65 (65%)	100 (100%)		0.0027*	
Exercise					
Non- Affected	**Affected**	**Total**		***P value* (P ≤ 0.05)**	
27 (27%)	73 (73%)	100 (100%)		0.0001*	
Animals in house					
No	**Animal in house**	**Total**		***P value* (P ≤ 0.05)**	
43 (43%)	57 (57%)	100 (100%)		0.1615NS	
*Significant difference at the 0.05 level by chi-square test NS: Non-significant difference					

3.2 Clinical characteristic of Asthma patients

Table (3-2) displays the clinical characteristics of patients were included in present study that including: Passive smoke, previous history of Covid19, Attacks required systemic Steroid, Food allergy, Comorbid condition (sinusitis, rhinitis, GERD) , exacerbation by Cold air or dry air,night time cough, affection by air pollutant, ICS usage duration, day time Wheezing, and Shortness of breath. According to passive smoke, the patients were divided into two groups: Non passive smoke (48%) and passive smoke (52%), however, the results of statistics showed non-significant differences (p=0 .6892) between groups. Regarding to previous

history of covid19 infection, the majority of patients were non- infected (85%), with statistically significant difference (p=0.0001). In the context of food allergy as sweets,spicy food and carbonated drinks, patients were distributed into two groups: without food allergy and with food allergy, the significantly (p=0.0003) higher percent of patients were within group of without food allergy (68%).Also, patients were divided according to Comorbid condtions (sinusitis, rhinitis, GERD) into: No Comorbid condtion and Comorbid condtion groups, there is a significantly (p=0.0001) higher percent of patients were within the Comorbid condtion group (77%).The distribution of patients according to Attacks required systemic Steroid included two groups ≤2times/6months(≤2/6m) and ≥4times /1year(>=4/1yr), however there is no significant difference (p=0.8415) between these groups. The presence or absence of night time cough also was recorded, where the majority (97%) of patients found with cough at night with significant differences (p=0.0001).Also no significant differences (p=0.6892) were reported in patients according to exacerbations by Cold or dry air while a significant exacerbations (p=0.0027) were reported to Air pollutant as fumes or dust(65% of patients were in affected group, while 35% in non affected group).

Day time symptoms as wheeze and shortnes of breath were present in 100% of patient .

Table (3-2): Clinical characteristic for Patients

Passive smoke			
Non passive smoke	**Passive smoke**	**Total**	***P value* (P ≤ 0.05)**
48 (48%)	52 (52%)	100 (100%)	0 .6892^{NS}
Previous history of Covid19 infection			
Non infected	**Infected**	**Total**	***P value* (P ≤ 0.05)**
85 (85%)	15 (15%)	100 (100%)	0.0001*
Food allergy			
Without food allergy	**With Food allergy**	**Total**	***P value* (P ≤ 0.05)**
68 (68%)	32 (32%)	100 (100%)	0.0003*
Comorbid condtions (sinusitis, rhinitis, GERD)			
No Comorbid condition	**Comorbid condition**	**Total**	***P value* (P ≤ 0.05)**
23 (23%)	77 (77%)	100 (100%)	0.0001*
Exacerbation of asthma that's required systemic steroid			
<= 2/6m	**>=4/yr**	**Total**	***P value* (P ≤ 0.05)**
49 (49%)	51 (51%)	100 (100%)	0.8415^{NS}
night time Cough			
Without cough	**With cough**	**Total**	***P value* (P ≤ 0.05)**
3 (3%)	97 (97%)	100 (100%)	0.0001*
Exacerbation by Cold or dry air			
Exacerbation	**No Exacerbation**	**Total**	***P value* (P ≤ 0.05)**
52 (52%)	48 (48%)	100 (100%)	0.6892^{NS}
ICS duration			
<3 year	**>3 year**	**Total**	***P value* (P ≤ 0.05)**
5 (5%)	95 (95%)	100 (100%)	0.0001*
Exacerbation byAir pollutant			
Non affected	**Affected**	**Total**	***P value* (P ≤ 0.05)**
35 (35%)	65 (65%)	100 (100%)	0.0027*
Day time Wheezing			
100 (100%)			----------
Day time Shortness of breath			
100 (100%)			----------
*Significant difference at the 0.05 level by chi-square test. NS: Non-significant difference			

3.3 Drug consumption of Asthmatic Patients

Table (3-3) shows the types of drugs consumed by Asthmatic patients. Patients were divided into different groups according to the type of treatment used. According to usage pattern of SABA, patients were divided into two groups:>2days per week (moderate asthma) (51%) and several times daily (sever asthma) (49%), however no significant (p=0.8415) differences were recorded. Depending on Inhalor beclomethasone or budesonide dose, patients were distributed into patients taken medium dose (100-400 **µg**) and patients take high dose steroid (>400 **µg**), A significant statistical association were present (p=0.0001) ,the patients taking high doses (73%) was higher than those taken medium doses (27%). It is worth noting that all patients within the study, 100%, were taking Montelucast.

Table (3-3): Drug conception from Patients:

SABA usage*			
>2 days/week	**Several times daily**	**Total**	***P value* (P ≤ 0.05)**
51 (51%)	49 (49%)	100 (100%)	0.8415^{NS}
Inhaled steroid usage			
Medium dose steroid (100-400) µg	**High dose steroid (>400) µg**	**Total**	***P value* (P ≤ 0.05)**
27 (27%)	73 (73%)	100 (100%)	0.0001^{*}
Montelukast			
100 (100%)			---------
***SABA**: **Short-Acting Beta Agonists**			

3.4 Assessment of laboratory markers in patients according to sex

Table (3-4) displays the assessment of Lab. Markers in Asthma patient according to sex, the statistical analysis revealed that total .IgE significantly (p=0.004) increased in male (557.2923±52.41625) compared with female patients (305.9336±52.28526), while the other markers showed non-significant (p>0.05) differences in the distribution of laboratory markers.

Table (3-4): Assessment of Lab. Markers in patients according to sex

Lab. parameters	Concentration (10^3\µL) Mean ± Std. Deviation		P value ($p \leq 0.05$)
	Male	**Female**	
Lymphocytes	4.5672 ± 0.32215	4.2797 ± 0.52921	0.630^{NS}
Neutrophil.	6.85807± 2.42306	6.3200 ± 0.43519	0.343^{NS}
Eosinophil	0.7529 ±0 .10731	0.6848 ±0.08183	0.689^{NS}
t .IGE	557.2923 ± 52.41625 *	305.9336 ± 52.28526	0.004^{*}
***Significant difference under p ≤ 0.05 by T-test NS: Non-significant difference**			

3.5 Assessment of laboratory markers in patients according to Age categories

Table (3-5) illustrates impact of age on the levels of Lab. Markers in Asthma patient. Current study recorded that only Lymphocytes. showed significant (p=0.000) differences, their levels were decreasing by age; where it increased in 5-8 y age category and significantly decreased in 9-12 y and 13-16 y age categories. On the other hand, the remaining markers showed in-significant (p>0.05) differences.

Table (3-5): Assessment of Lab. Markers in patients according to Age categories

Lab. parameters	Age group Mean ± Std. Deviation (10^3\μL)			P value ($p \leq 0.05$)
	5-8 y	**9-12 y**	**13-16 y**	
Lymphocytes	5.6491 ±0.39157 a	3.0259 ± 1.00749 b	2.721 ± 0.3817 b	0.000 *
Neutrophil.	6.5033 ± 2.376	18.7070 ± 9.303	5.680 ± 1.659	0.064 NS
Eosinophil	0.7793 ±0.12274	0.7304 ± 0.09951	0.5650 ± 0.140	0.629NS
t .IGE	470.1934 ± 55.3823	589.4426 ± 78.68812	326.321± 89.931	0.124NS
Total No.	57	27	16	

Different small letters refer to significant between-groups comparison
***Significant difference under p ≤ 0.05 by One way – ANOVA**
NS: Non-significant difference

3.6 Assessment of laboratory markers in patients according to BMI categories

Table (3-6) summarizes the effects of BMI on the Lab. markers in Asthma patients. The statistical analysis revealed a significant (p=0.000) increase in the concentration of lymphocyte in Underweight category compared with others BMI categories. Also, the concentrations of Eosinophil significantly (p=0.031) increased in Underweight category versus to other categories.

Neutrophils and t .IgE showed non-significant differences, despite a trend toward significant differences (p=0.055) were recorded in distribution of neutrophils.

Table (3-6): Assessment of Lab. Markers in patients according to BMI categories

Lab. Markers	BMI Mean ± Std. Deviation (10^3\µL)				P value ($p \leq 0.05$)
	Underweight	**Normal**	**Overweight**	**Obesity**	
Lymphocytes	7.1641± 2.694 a	3.6182 ± 1.268 b	4.4692 ± 1.6436 b	3.2133 ± 0 .394 b	0.000*
Neutrophil.	6.5659 ±2 .555	6.048 ± 1.319	6.5659 ± 2.659	5.1256 ± 1.106	0.055NS
Eosinophil	1.1545 ± 0.2854a	0.6113 ±0.069 b	0.7062 ± 0.096 b	0.4856 ± 0.132 b	0.031*
t .IGE	627.486± 89.017	399.614 ± 54.31	578.73 ± 97.487	470.05 ± 148.529	0.125NS
Total No.	22	56	13	9	

Different small letters refer to significant between-groups comparison
***Significant difference under $p \leq 0.05$ by One way – ANOVA**
NS: Non-significant difference

3.7 Percentage of bacterial growth according to Bacterial Gram stain

Figure (1) displays that bacterial Gram positive percent was 16%, while the percent of Gram negative was 25%.

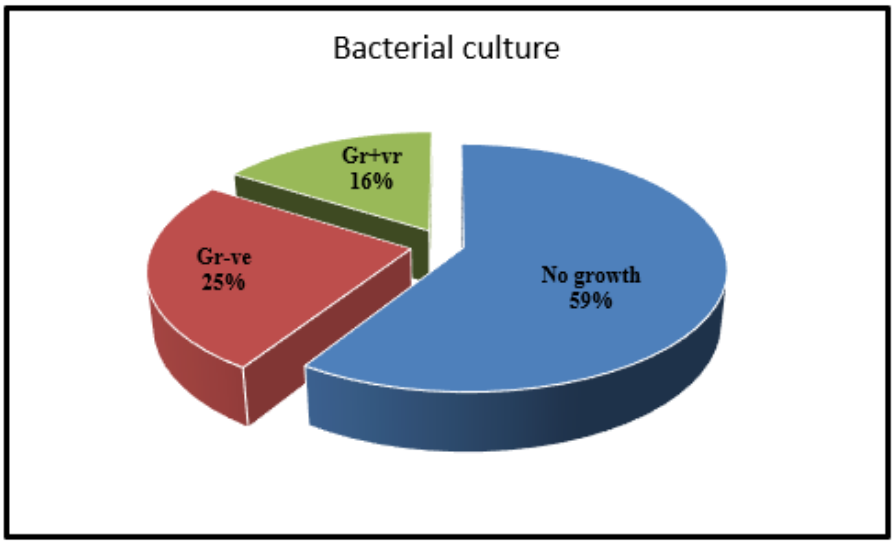


Figure (3.1): Percent of bacterial growth

3.7.1 Evaluation of Lab. parameters in patients according to Bacterial Gram stain

Table (3-7) shows the evaluation of Lab. markers in Asthma patients according to Bacterial Gram stain. The results of statistical analysis using one way – ANOVA, revealed that only Lymphocyte and Eosinophil showed a significant differences (p=0.000 and 0.001, respectively). The both levels of Lymphocyte and Eosinophil showed a significant decrement in gram negative bacteria. Neutrophils and t .IGE also decreased in gram negative bacteria, but the differences were in-significant.

Table (3-7): Evaluation of Lab. parameters in patients according to Bacterial Gram stain

Gram stain bacteria	Lab. parameters Mean ± Std. Deviation (10^3\μL)			
	Lymphocyte	**Neutrophils.**	**Eosinophil**	**t .IGE**
No growth	5.244±0 .362 [a]	10.84 ±4.0733	0.949 ± 0.117 [a]	507.476± 53.633
Gr.-ve B.	2.513 ±0.210 [b]	7.626 ±.57365	0.280 ±0.062 [c]	348.202± 78.817
Gr.+ve B.	4.686 ±0.728 [a]	8.512 ±.62901	0.635 ±0.021 [b]	580.685± 103.542
Total	4.4725 ± 0.2725	9.666 ±3.378	0.731 ±0.028	479.371±41.17581
P value ($p \leq 0.05$)	0.000*	0.674NS	0.001*	0.151NS

Different small letters refer to significant between-groups comparison
***Significant difference under p ≤ 0.05 by One way – ANOVA**
NS: Non-significant difference

3.8 Bacterial culture

Figure (2) illustrates the results of bacterial cultures obtained by the present study, the results of statistical analysis using Chi-square- χ^2 indicated highly significant differences (p<0.0001) among the bacterial isolates, where higher percentage of cultures had no growth (59%), the

highest bacterial isolate that appeared was *streptococcus pneumoniaee* (11%), while *Proteus spp*., *E. coli*, *Enterobacter cloacae*, *Staphylococcus aureus, klebsiella oxytoca*, and *Acinetobacter baumannii* were the least frequent, accounting for 2%.

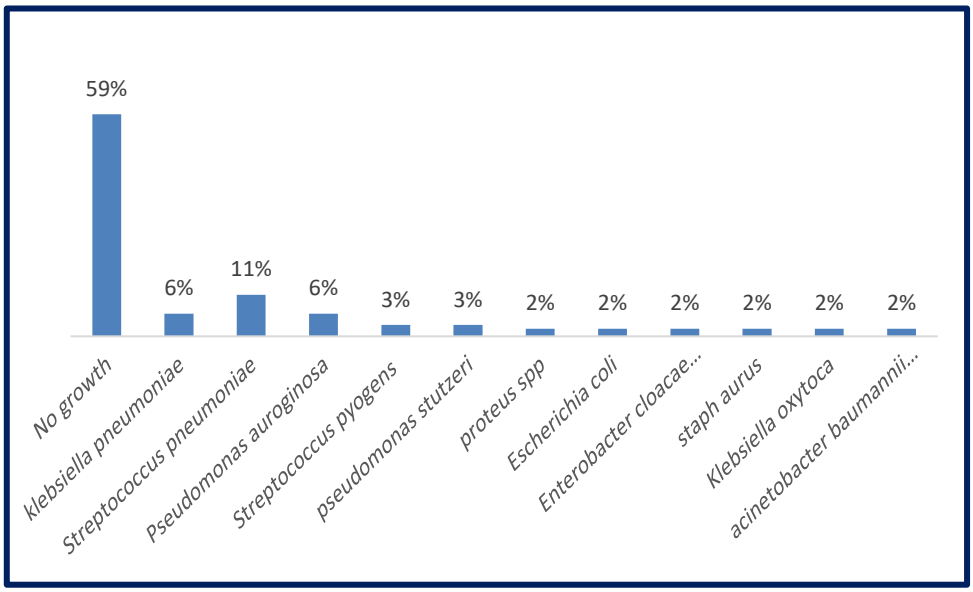


Figure (3.2): Bacterial culture results

3.8.1 Evaluation of Lab. markers in Asthma patients according to Bacterial growth

Table (3-8) displays the evaluation of Lab. markers in Asthma patients according to Bacterial growth. According to the results of statistical analysis using one way – ANOVA, only Lymphocytesocyte and t .IgE showed a significant differences (p=0.002 and 0.048, respectively); The highest concentration of lymphocyte was found in patients from whom *Staphylococcus aureus* were isolated as compared with remaining bacterial isolates. Regarding to (t .IgE), the highest concentration was recorded in patients from whom *Staphylococcus aureus* and *Enterobacter cloacae* complex were isolated, as compared with remaining bacterial isolates.

Table (3-8): Evaluation of Lab. markers in patients according to Bacterial type

Bacterial growth	Lab. markers Mean ± Std. Deviation (10^3\µL)			
	Lymphocytes	**Neutrophil.**	**Eosinophil**	**t .IGE**
No growth	5.244 ± 2.783 b	10.84 ±4.0733	0.949 ±0 .117	507.476 ± 53.633 c
Klebsiella pneumoniaee	2.135 ± 1.016 c	8.103 ± 0.823	0.358 ± 0.168	414.925 ± 186.021c
Streptococcus pneumoniaee	4.285 ± 2.574 b	7.745 ± 0.255	0.664 ±0.117	426.8155 ± 97.509 c
Pseudomonas aeruginosa	2.766 ± 1.31 c	9.146 ±0.396	0.363 ±0.191	460.2717 ± 198.363 c
Streptococcus pyogens	2.97 ± 1.5108 c	10.546 ± 3.320	0.316 ±0.226	731.9967 ± 268.003 b
pseudomonas stutzeri	3.26 ± 1.327 c	7.3467 ±0.426	0.106 ± 0.013	123.1767 ± 41.286 d
Proteus spp	3.63 ± 0.102 c	6.88 ±0.0125	0.09 ±0.102	89.4500 ±2.102 d
Escherichia coli	2.90 ±0 .011 c	11.50 ± 0.024	0.20 ±0.028	241.5400 ±3.002 d
Enterobacter cloacae complex	1.63 ± 0.622 c	4.875 ± 3.205	0.28 ±0.0050	1000.0 ± 32.0010 a
staph aureus	9.48 ±2 .0123 a	9.68 ± 0.123	0.96 ±0.101	1200.0±1.01 a
Klebsiella oxytoca	1.83 ± 1.021 c	8.31 ±0.033	0.51 ± 0.015	79.09 ±2 .121 d
Acinetobacter baumannii complex	1.83 ±0.1402 c	0.99±0.1002	0.090 ± 0.0325	132.1±12 .024 d
Total	4.47 ± 2.783	13.062 ± 8.032	0.7318 ±0.078	479.3711± 41.175
P value ($p \leq 0.05$)	0.002^{*}	1.000^{NS}	0.186^{NS}	0.048^{*}

Different small letters refer to significant between-groups comparison
***Significant difference under $p \leq 0.05$ by One way – ANOVA**
NS: Non-significant difference

3.9 The relation between asthma control and bacterial growth

In Table (3-9) the results of statistical analysis included two parts, between-groups (Partially controlled asthma and Uncontrolledly asthma) comparison as well as within-group comparison (within- Partially controlled and within- Uncontrolledly). At the level of between-groups comparison, the culture with no growth significantly (p=0.0063) increased in patients had Not- well control (67.8%) as compared with Uncontrolledly

control (32.2%). *Klebsiella pneumoniaeStreptococcus pneumoniae*and *Pseudomonas auroginosa* were significantly (p=0.0001, 0.0067, and 0.0001, respectively) increased in patients had Partially controlled Vs. Uncontrolledly. While other bacterial isolates showed non-significant differences. Regarding to within group comparison, within- Partially controlledcontrol as well as within- Uncontrolledly control showed significant differences (p=0.000) in the appearing of bacterial isolates as explained in Table (3-9)

Table (3-9): The association between level of control and bacterial growth

Bacterial growth	Level of control No. (%)			P value (p ≤ 0.05)
	Partially controlled	**Uncontrolledly**	**Total**	
No growth	40 (67.8%)*	19 (32.2%)	59	0.0063*
Klebsiella pneumoniaee	1 (16.7%)	5 (83.3%)*	6	0.0001*
Streptococcus pneumoniaee	1 (9.1%)	10 (90.9%)*	11	0.0067*
Pseudomonas auroginosa	1 (16.7%)	5 (83.3%)*	6	0.0001*
Streptococcus pyogens	0 (0%)	3 (100%)	3	-----
pseudomonas stutzeri	0 (0%)	3 (100%)	3	-----
proteus spp	0 (0%)	2 (100%)	2	-----
Escherichia coli	2 (100%)	0 (0%)	2	-----
Enterobacter cloacae complex	0 (0%)	2 (100%)	2	-----
staph aurus	0 (0%)	2 (100%)	2	------
Klebsiella oxytoca	0 (0%)	2 (100%)	2	------
acinetobacter baumannii complex	0 (0%)	2 (100%)	2	------
Total	45 (45%)	55 (55%)	100	
P value (p ≤ 0.05)	0.000*	0.000*		

***Significant difference under p ≤ 0.05 by Chi-square test**
NS: Non-significant difference

3.10 The association of SABA usage with bacterial growth

In Table (3-10) the results of statistical analysis included two parts, between-groups (2days/week usage (moderate) and several times daily (sever)) comparison as well as within-group comparison (within- 2 days/week and within-several times daily). Regarding to between-groups comparison, the results showed only significant (p=0.0001) differences in *klebsiella pneumoniaee* as well as *Pseudomonas auroginosa*, the higher percentages were in several times daily use of SABA; the other bacterial growth showed in-significant differences. While the result of both within-Daily and within- several times daily comparisons demonstrated significant (p=0.042 and 0.004, respectively) differences as explained by Table (3-10).

Table (3-10): Associated SABA used with bacterial growth

Bacterial growth	SABA use No. (%)			P value ($p \leq 0.05$)
	>2days/wk (moderate)	**Several times daily (sever)**	**Total**	
No growth	26 (44.1%)	33 (55.9%)	59	0.3621^{NS}
klebsiella pneumoniaee	2 (33.3%)	4 (66.7%)*	6	0.0001^{*}
Streptococcus pneumoniaee	6 (54.5%)	5 (45.5)	11	0.7630^{NS}
Pseudomonas auroginosa	2 (33.3%)	4 (66.7%)*	6	0.0001^{*}
Streptococcus pyogens	0 (0%)	3(100)	3	-------
Pseudomonas stutzeri	3(100)	0 (0%)	3	-------
Proteus spp	2 (100)	0 (0%)	2	-----
Escherichia coli	2 (100)	0 (0%)	2	-----
Enterobacter cloacae complex	2 (100)	0 (0%)	2	-----
staph aurus	0 (0%)	2(100%)	2	------
Klebsiella oxytoca	2 (100)	0 (0%)	2	------
acinetobacter baumannii complex	2 (100)	0 (0%)	2	------
Total	51 (51%)	49 (49%)	100	
P value ($p \leq 0.05$)	0.042^{*}	0.004^{*}		

***Significant difference under $p \leq 0.05$ by chi-square test**
NS: Non-significant difference

3.11 Bacterial growth percentage according to type of sample

In Table (3-11) the results of statistical analysis included two parts, between-groups (Sputum and Cough) comparison as well as within-group (within- Sputum group and within- Cough group) comparisons. No growth isolates appeared more frequently (93.2%) in cough samples than in sputum samples (6.8%), p=0.0001. *klebsiella pneumoniaee* appeared more frequently (66.7%) in sputum samples than in cough samples (33.3%), p=0.0001. *Streptococcus pneumoniaee* appeared more frequently (72.7%) in cough samples than in sputum samples (27.3%), p=0.0001. *Pseudomonas aeruginosa* appeared more frequently (83.3%) in cough samples than in sputum samples (16.7%), p=0.0001. *Pseudomonas stutzeri* appeared more frequently (66.7%) in sputum samples than in cough samples (33.3%), p=0.0001. While other bacterial isolates showed non-significant differences (p>0.05). Regarding the result of both within-Sputum and within-Cough comparisons demonstrated significant (p=0.000 and 0.002, respectively) differences as explained by Table (3-11).

Table (3-11): Bacterial growth percentage according to type of sample

Bacterial growth	Type of sample No. (%)			P value (p ≤ 0.05)
	Sputum	**Cough swab**	**Total**	
No growth	4 (6.8%)	55(93.2%)*	59	0.0001*
Klebsiella pneumoniaee	4 (66.7%)*	2 (33.3%)	6	0.0001*
Streptococcus pneumoniaee	3 (27.3%)	8(72.7%)*	11	0.0001*
Pseudomonas aeruginosa	1(16.7%)	5 (83.3%)*	6	0.0001*
Streptococcus pyogens	0(0%)	3 (100%)	3	-----
Pseudomonas stutzeri	2 (66.7%)*	1(33.3%)	3	0.0001*
Proteus spp	0(0%)	2 (100%)	2	-----
Escherichia coli	0(0%)	2 (100%)	2	-------
Enterobacter cloacae complex	1 (50.0%)	1(50.0%)	2	1.000NS
Staph aureus	0(0%)	2 (100%)	2	-------

Klebsiella oxytoca	0(0%)	2 (100%)	2	-------
Acinetobacter baumannii complex	2 (100%)	0 (0%)	2	-----
Total	17 (17%)	83 (83%)	100	
P value ($p \leq 0.05$)	0.000*	0.002*		
***Significant difference under $p \leq 0.05$ by chi-square test NS: Non-significant difference**				

3.12 Correlation analysis

Correlation analysis using Pearson's correlation coefficients was applied among the lab. markers; the results are illustrated in Table (3-12).

t.IgE showed a significant positive correlation with Lymphocytesocyte (p=0.045) and Eosinophil (p=0.014). Lymphocytesocyte had a significant positive correlation with Eosinophil (p=0.015).

Table (3-12): Correlation between Lab. markers in patients

Lab. markers		t .IGE	Lymphocyte socyte	Neutrophil	Eosinophil
t .IGE	**Pearson Correlation**	1	0.201	0.189	0.246
	Sig. (2-tailed)		0.045*	0.060	0.014*
	N		100	100	100
Lymphocytesocyte	**Pearson Correlation**		1	-0.079-	0.244
	Sig. (2-tailed)			0.432	0.015*
	N			100	100
Neutrophil	**Pearson Correlation**			1	-0.051-
	Sig. (2-tailed)				0.617
	N				100
Eosinophil	**Pearson Correlation**				1
	Sig. (2-tailed)				
	N				100
*. Correlation is significant at the 0.05 level (2-tailed).					

Chapter Four

Discussion

Discussion

4.1 Demographic characteristics of the study sample

Current study found that the majority of participants were in the 5-8 year age group (57%), and the lowest at 13-16 year age group (16%). This age distribution aligns with the findings (Li *et al.*, 2020), who reported a higher prevalence of health issues in younger pediatric populations, Children aged 5-8 years were with the highest prevalence estimate of 2.65% (95% CI = 2.31-3.12) and those aged 9-12 years were with the lowest (1.48%, 95% CI = 1.26-1.78). The significant P-value (0.0001) indicates a non-random distribution of participants across age groups, which may reflect developmental stages' impact on health. Age of school entry could be associated with a higher risk of infection due to an increase exposure rate.

The distribution of BMI categories—22% underweight, 56% normal weight, 13% overweight, and 9% obese—was statistically significant (P=0.0001). This distribution of asthma reported by a previous study (Black *et al.*, 2012) was overweight, moderate, and extreme obesity are associated with higher odds of asthma in children and adolescents, although the association varies widely with race/ethnicity

Urban (44%) and rural (56%) residency distributions did not show a significant difference (P=0.2301). This non-significant finding contrasts with the results of (McCrorie *et al.*, 2020), who observed significant health disparities between urban and rural children. The difference might be due to varying healthcare access or socioeconomic conditions in the study settings.

The sex distribution in the present study—69% male and 31% female—was significantly skewed (P=0.0001). This imbalance is similar to

that reported by past study (Chowdhury *et al.*, 2021), who found higher asthmatic male participation in his study, possibly due to higher healthcare-seeking behavior among males. In the present study, higher male rate may be due to more medical attention in community for males than females.

Family history was a significant factor (P=0.0027), with 65% of participants having a positive family history of health issues. This finding is supported by previous studies, such as (Frei, Heye and Roduit, 2022), which highlighted the genetic and environmental influences on pediatric health outcomes.

Exercise habits were significantly associated with asthma (P=0.0001), with 73% of participants reporting symptoms affection by exercise. This result aligns with Cordova-Rivera *et al.,* (2018) found in their study that patients with severe asthma generally had lower levels of physical activity than those with milder asthma or healthy individuals. Many patients with severe asthma were classified as physically inactive, engaging in limited physical exercise, while the same study reported that increasing physical activity and improving exercise capacity are key targets for interventions aimed at improving asthma management and quality of life for patients with severe asthma.

The presence of animals in the house did not show a significant association with (P=0.1615) although it is more than no animal group, which is inconsistent with the findings of (Ji *et al.*, 2022), who suggested that pet ownership's impact on health and there was significant association between pets and incidence of asthma.

4.2 Clinical characteristic for patients

Passive Smoke Exposure, the study found no significant difference in asthma prevalence between children exposed to passive smoke (52%)

and those not exposed (48%) (P=0.6892). This result disagree with the findings of (He *et al.*, 2020), who reported a strong association between passive smoke exposure and increased asthma risk in children. The discrepancy might be due to differences in sample size, geographic location, or assessment methods.

Regarding COVID-19 Infection**,** no significant association was observed between COVID-19 infection and asthma prevalence, with 15% of asthmatic children previously infected with COVID-19 (P=0.0001). This disaligns with (Hurst *et al.*, 2021), who noted higher asthma exacerbation rates in children affected previously by COVID-19. The findings emphasize the need for heightened monitoring and proper diagnosis of causative agent in society.

The frequency of asthma attacks requiring systemic steroids (<2 attacks in 6 months or ≥4 attacks in 1 year) showed no significant difference (P=0.8415). This finding is consistent with Bacharier *et al*., (2004), who also found no significant variation in asthma severity levels among different pediatrics, suggesting that systemic steroid requirements may not directly correlate with attack frequency in some populations.

Also no significant relationship was found between food allergies and asthma, with 32% of asthmatic children having food allergies (P=0.0003). This result in disagree with the study by Aba-Alkhail & El-Gamal, (2000), which demonstrated a higher prevalence of food allergies among asthmatic children. These findings highlight the importance of screening for causes of deviation from the fact of food allergies in asthmatic children.

Regarding Comorbid Conditions, The presence of comorbid conditions (sinusitis, rhinitis, GERD) was significantly higher among

asthmatic children (77%) compared to those without comorbidities (23%) (P=0.0001). This observation is in line with Porsbjerg & Menzies-Gow, (2017), who reported that comorbid conditions often exacerbate asthma symptoms and complicate management strategies.

A significant association was found between night time cough (97%) and asthma prevalence (P=0.0001), and a non-significant association between exposure to cold air or dry air and asthma (P=0.6892). These results partially align with (Hyrkäs *et al.*, 2014), who reported that cold air can trigger asthma symptoms, but the overall impact varies based on individual susceptibility and environmental factors.

Air pollution in the non-affected group was significantly higher than the affected group (P=0.0027). This finding disagrees with past study by Madaniyazi & Xerxes, (2021), which highlighted air pollutants as a critical factor in asthma exacerbations. It underscores the need for environmental interventions to reduce exposure to harmful pollutants, whoever differences in studies could be due to difference in geographical areas or sample size of studies.

Regarding Inhaled Corticosteroid (ICS) Duration, the duration of ICS use was significantly associated with asthma management, with 95% of children using ICS for more than 3 years (P=0.0001). This is consistent with Singanayagam & Johnston, (2020), who found that long-term ICS use is effective in controlling asthma symptoms and reducing exacerbations.

4.3 Drug Conception from Patients

The division of patients into2 day per week usage of SABA (moderate asthma) and several times daily (severe) SABA users revealed no significant differences (p=0.8415). This finding disaligns with research by (Noorduyn *et al.*, 2022) which found that the frequency of SABA use

associated with increased risk of severe exacerbations and can be used to identify patients at a higher risk for severe exacerbations. These studies emphasize that SABA use is often a marker of symptom management rather than a sole indicator of asthma control.

A significant difference was observed in the ICS dosage, with a higher percentage of patients using high doses (>400) compared to medium doses (27% vs. 73%, p=0.0001). This aligns with findings from studies like those by (Wang, Zhou and Xie, 2019) found Lung function was significantly improved in the patient group receiving low- to medium-dose ICS. The significant difference in dosages highlights the varying needs for treatment intensity based on disease severity.

The study notes that all patients were taking Montelukast. This is consistent with current guidelines and practices where Montelukast, a leukotriene receptor antagonist, is commonly prescribed as an add-on therapy for asthma management. Previous study by (Lee and Kim, 2020) support the widespread use of Montelukast due to that montelukast has significantly contributed to asthma control and has been critical for reducing asthma severity, especially early wheezing and disease control. However, it is also essential to recognize that while Montelukast is beneficial, it may not be sufficient alone for some patients, and its effectiveness can vary.

4.4 Sex, age, and BMI Categories differences with laboratory markers

In present study, total IgE (t.IgE) levels were significantly higher in male patients compared to females, whereas other parameters like lymphocyte, neutrophils, and eosinophile did not show significant sex differences. This finding is consistent with the study by Brakhas *et al*., (2016), which also reported there is a significant elevate in mean serum t-IgE in patients through sex groups compared to the control group, the mean

of serum t-IgE levels increased in asthmatic males 506.025 ± 138.7 IU/ml compared to asthmatic females. At the same time, in contrast with the same study, a significant difference was found in the percentage count of eosinophile in (cases) allergic asthma. As well as, this study in line with Dogru & Mutlu, (2016) found there was no difference between the groups concerning sex, the mean neutrophil-lymphocytesocyte ratio (NLR) was 2.07 in the case group and 1.77 in the control group

Current results showed a significant decrease in lymphocytesocyte levels with increasing age, particularly marked in the 9-12 and 13-16 years categories. This trend aligns with the findings of Weerkamp et al., (2005), who demonstrate that distribution of thymocyte subsets changes with age and correlates with age-related fluctuations of T-lymphocytesocyte counts in peripheral blood.

Significant differences in lymphocytesocyte and eosinophilophil levels were observed across different BMI categories, with underweight children showing higher levels of these markers. This is in agreement with the study by Rhee *et al.,* (2018) which reported blood neutrophil count was significantly correlated with BMI but not with symptoms and severity of asthma, possibly due to underlying nutritional deficiencies or different immune response patterns.

4.5 Bacterial Growth results

The analysis of bacterial growth revealed that lymphocytesocyte and eosinophilophil levels were significantly lower in patients with gram-negative bacterial infections compared to those with gram-positive or no bacterial growth. However, this finding contrasting evidence from Sumardi *et al.*, (2021) suggests that lymphocytesocyte counts in patients infected with gram-positive bacteria were significantly lower (239–742/mm^3) versus (573–1,138/mm^3) in those infected with gram-negative bacteria with a

significant association at p-value > 0.007, and absolute neutrophil count was higher in gram-positive bacterial infection (11,409–24,080 mm^3) versus gram-negative bacterial infection (10,102–20,394 mm^3).

The present study analyzed bacterial cultures from asthma patients, revealing a high prevalence of no bacterial growth (59%) and identifying *Streptococcus pneumoniaee* as the most common isolate (11%). Other bacterial isolates, such as Proteus spp., *E. coli, Enterobacter cloacae*, *Staphylococcus aureus, Klebsiella oxytoca,* and *Acinetobacter baumannii*, were found to be infrequent, each accounting for only 2% of cultures.

This finding is partially in line with research by (Ramesh, Hemalatha and Thaliath, 2023) found 74 (66 .07%) were culture positive and 38 (33.93 %) were culture negative, and from 74 culture positive distributed as (63.51 %) were *streptococcus*, (18.92%) were *Klebsiella,* (14 .86%) were *Pseudomonas* and (2%) were MRSA. This can be attributed to the chronic inflammation and altered immune response in these patients, which may not always be accompanied by detectable bacterial pathogens.

4.5.1 Bacterial specific Growth and Laboratory Markers

In current study, lymphocyte levels were significantly different across bacterial groups, with the highest levels observed in children with *S. aureus* (9.48 ±2 .0123) and the lowest in those with *Enterobacter cloacae complex* (1.63 ± 0.622) and *Klebsiella oxytoca* (1.83 ± 1.021). These findings are consistent with (Cheng *et al.*, 2020), who found that gram-negative bacterial infections, such as those caused by *Enterobacter* are associated with lower lymphocyte levels due to immune suppression.

The study showed no significant differences in neutrophil levels across bacterial groups (p = 1.000), which aligns with the findings of (Crisford, Sapey, Geraint B Rogers, *et al.*, 2021),who also reported non-significant

differences in neutrophil levels among different bacterial infections in asthmatic patient. This indicates that neutrophil response might not be significantly influenced by the type of bacterial infection in asthma.

Eosinophil levels did not show significant differences across bacterial groups ($p = 0.186$), which contrasts with Son et al., (2020) who found significant variations in eosinophil counts among different bacterial infections, particularly noting higher levels in children with gram-positive bacterial infections. These findings suggest that eosinophil response might be more variable and influenced by other factors such as underlying atopic conditions.

Total IgE levels varied significantly across bacterial groups ($p = 0.048$), with the highest levels in children with *Staphylococcus aureus* (1200.0 ± 1.01) and the lowest in those with *Klebsiella oxytoca* (79.09 ± 2.121). This is in agreement with Chiu *et al.*, (2021), who reported elevated IgE levels in children with gram-positive bacterial infections like *Staphylococcus aureus*.

4.5.2The association between management and bacterial colonization.

The present study examined bacterial culture results in asthma patients with different levels of disease control, specifically comparing those with "Not well" control to those with "Uncontrolledly" control. The analysis revealed several significant findings: a higher proportion of cultures with no bacterial growth in the "Not well" control group compared to the "Uncontrolledly" control group ($p=0.0063$); increased frequencies of *Klebsiella pneumoniaeStreptococcus pneumoniae*and *Pseudomonas aeruginosa* in the "Uncontrolledly" controlled group compared to the "Not well" controlled group ($p=0.0001$, $p=0.0067$, and $p=0.0001$, respectively); and significant within-group differences in bacterial isolates for both control categories ($p=0.000$). This discussion contextualizes these findings

with similar and differing studies to provide a comprehensive understanding.

The observation that cultures with no bacterial growth were more prevalent in the "Not well" control group (67.8%) compared to the "Uncontrolledly" control group (32.2%) is intriguing. For instance, study by (Green *et al.*, 2014) found that Airway colonization with potentially pathogenic micro-organisms in asthma is associated with more severe airway obstruction and neutrophilic airway inflammation, these differences are potentially due to chronic inflammation or a less acute bacterial infection. No growth result doesn't exclude the bacterial infection possibility as it could be non-culturable bacteria. In addition, other non bacterial infections as viral infections could affect the state of disease control.

Conversely, the increased detection of *Klebsiella pneumonia Streptococcus pneumonia* and *Pseudomonas aeruginosa* in patients with "Uncontrolledly" control is consistent with studies highlighting that poor asthma control is often associated with a higher incidence of specific bacterial pathogens. For example, study (Hewitt *et al.*, 2016) observed virus-induced exacerbations of asthma and COPD and virus–bacteria interactions such as *Streptococcus pneumonia* HRV and RSV, supporting the finding that more severe asthma control is associated with a greater prevalence of these pathogens.

Regarding Within-Group Comparison: Bacterial Diversity, the significant differences in bacterial isolates within both the "Not well" and "Uncontrolledly" controlled groups (p=0.000) suggest variability in microbial profiles depending on the level of asthma control. This variability is in line with studies by (Guo *et al.*, 2021) which indicate that bacterial populations in the respiratory tract can change significantly with the control

status of chronic respiratory diseases. This study contrasts with study focusing on different conditions, such as by (Combes *et al.*, 2007), which examined ventilator-associated pneumonia and severe infections, the present findings highlight specific patterns in asthma patients. For instance, while these studies reported a higher frequency of gram-negative bacteria in severe infections, the present study's focus on asthma reveals a more nuanced relationship between bacterial types and disease control. The lower prevalence of gram-negative bacteria such as *Pseudomonas aeruginosa* in other studies compared to their higher prevalence in poorly controlled asthma highlights differences in pathogen profiles across different respiratory conditions.

Additionally, research by (Sandrock and Norris, 2015) observed that in patients with severe asthma or exacerbations, there is often an increased presence of specific pathogens compared to those with stable asthma. This supports the present study's findings that patients with "Uncontrolledly" control have a higher incidence of certain bacteria, reflecting a broader trend of more severe infections correlating with worse asthma control.

4.5-3 The association of SABA usage with bacterial growth

This study analyzed the bacterial growth in patients with different levels of asthma control and the influence of SABA usage. The findings showed significant differences in bacterial specific growth between patients with moderate and severe asthma.

The significant increase in *Klebsiella pneumoniaee* and *Pseudomonas aeruginosa* in patients with severe asthma which is consistent with the observations by (Garcia-Clemente *et al.*, 2020) who found these *Pseudomonas aeruginosa* has been related to more severity and poor prognosis in people with CF, bronchiectasis, and probably in COPD.

This aligns also with the findings of a study by (Crisford, Sapey, Geraint B Rogers, *et al.*, 2021) which also reported a higher incidence of bacterial growth in patients with poorly controlled and severe asthma. Both studies highlight the potential impact of asthma severity on bacterial colonization, suggesting that increased bacterial growth could exacerbate asthma symptoms or result from higher antibiotic usage in severe cases.

4.5.4 Bacterial growth percentage according to type of sample

The analysis revealed significant differences in bacterial growth based on the type of respiratory sample. Sputum samples showed higher frequencies of *Klebsiella pneumonia* and *Pseudomonas stutzeri* compared to cough samples. Also, the cough sample reveal more positive culture results compared to sputum. In addition, a significant number of S. *pneumonia* & *P. aeruginosa* were obtained by cough samples. This could highlight the importance of cough samples in the diagnosis of respiratory tract infections, especially in children with difficult sputum samples obtaining.

García-Vázquez et al., (2004) found in their study that sputum cultures provided useful in diagnosing community-acquired pneumoniae but had limitations due to contamination with upper respiratory flora. Bronchoalveolar lavage (BAL) and pleural fluid cultures were more specific, yielding higher rates of pathogenic bacteria like *Streptococcus pneumoniaee* and *Legionella* in severe cases. (Murray & Hill ;2009):

Murray & Hill, (2009) focused in their study on bacterial colonization in bronchiectasis and compares bacterial growth patterns in different respiratory sample types, including sputum and bronchoalveolar lavage. Found bacteria like *Haemophilus influenzae* and *Streptococcus pneumoniaee* often colonize sputum samples in bronchiectasis. However, culture results vary based on the quality of the sputum sample, often

necessitating the use of Bronchoalveolar lavage (BAL) for accurate detection of pathogens.

4.6 Correlation Analysis

Correlation analysis showed significant positive correlations between t.IgE and both lymphocytes and eosinophiles as well as between lymphocyte and eosinophiles themselves. These correlations are in line with the findings of Ahmed & Hussein, (2023) which also reported similar relationships, suggesting that positive correlations between t.IgE and lymphocyte. While Jassim & Al-Kazaz, (2023) found an association between t.IgE with eosinophile. Both IgE and eosinophil cells increased in allergic atopic asthma. Atopic asthma children infected with viruses could be presented with higher counts of lymphocyte.

Conclusions

&

Recommendations

Conclusion

1- The bacterial culture analysis showed that Gram-negative bacteria are more prominent and present in a considerable percentage of patients.

2- *Streptococcus pneumoniaee* is the most common bacterial isolate. There was also a high significant association between poor asthma control and the presence of specific bacterial isolates such as *Klebsiella pneumoniaeStreptococcus pneumoniae*and *Pseudomonas aeruginosa*.

3- Laboratory marker analysis identified elevated levels of lymphocyte and immunoglobulin E (IgE) in patients with bacterial growth, especially in those with *Staphylococcus aureus* infections, which may indicate an immune response to these pathogens.

4- Cough samples revealed the highest negative growth result compared to sputum samples, but the positive bacterial growth is higher in cough than in sputum samples. This highlighted the role of cough samples in the diagnosis of RTI especially in patients with difficulties in obtaining sputum samples as children.

5- The majority of patients were young children (5-8 years),normal weight, predominantly males,positive family history and affected by exercise , comorbid conditions such as sinusitis, rhinitis & GERD in most patients and air pollutants

6- There is no significant association with obesity,residence,animals in the house, ,passive smoking ,past history of COVID-19 infection,food allergy,systemic steroid use ,cold or dry air

7- Significantly common symptoms as day time whease,shortness of breath and night time cough despite widely used drugs among patients.

8- Drug consumption patterns indicated widespread use of Montelukast, and high-dose inhaled corticosteroids (ICS).

Recommendation

1. **Address Bacterial Infections:** Regular bacterial screening should be included in the management of asthma, especially for patients with frequent exacerbations or poor disease control. Appropriate antibiotic treatment and infection prevention strategies should be incorporated into asthma management plans.
2. **Focus on Allergy and Immune Responses:** With elevated levels of IgE and lymphocyte observed in patients with bacterial infections, a large sample study focusing on the association between type of bacterial growth and immune response in asthma. Also, an association between asthma phenotypes and bacterial isolate study is highly recommended to understand the interaction between immune response and infection.
3. **Regular Monitoring of Comorbid Conditions:** Asthma patients often have comorbid conditions like sinusitis and rhinitis. Routine screening and treatment of these conditions should be integrated into asthma care to reduce symptom burden and improve patient outcomes.
4. Pay attentions of ministry of health toward wide spread usage of drugs of asthma in the society that can be replaced by more safe less cost and side effects drugs

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Appendix

Appendix (1)

QUESTIONNAIRE

File Number: . . . . . .Date : . . . . . .

Patient Name: . . . . phone number . . . .Age : . . . . . .

Sex male ☐ female ☐ Address : . . . . . .

Weight : . . . . . kg Height:. . . . . .cm (BMI=WT(KG)/(HT M)2

Asthma Severity:

a- Mild ☐

b- Moderate ☐

c- Severe ☐

History : Personal (hx.)

Eczema Yes ☐ NO ☐ shortness of breath Yes ☐ NO ☐

Allergic Rhinitis (AR) Yes ☐ NO ☐ wheezing Yes ☐ NO ☐

Allergic conjunctivitis Yes ☐ NO ☐

cough Yes ☐ NO ☐

Food allergy : Yes ☐ NO ☐ Type : . . . . . .

Attacks/year: 3 & more ☐ < 3 ☐

Response to bronchodilator: good ☐ bad ☐

Nebulizer use: yes ☐ no ☐

Previous history of Covid19 yes ☐ No ☐

family hx. Of asthma

yes ☐ ☐

Aggravating factors :

Viral infection yes ☐ no ☐ cold air yes ☐ no ☐

Exercise yes ☐ no ☐ dust exposure yes ☐ no ☐

Playing yes ☐ no ☐ fume yes ☐ no ☐

Passive smoking yes ☐ no ☐

Animal in the house:

Cat…….. dog ……chicken…….Birds…….Cows…… sheep……..

Treatment:

Montelucast ……☐……. ICS……☐……

Level of control:

Well ☐ Partially controlled ☐ Uncontrolledly ☐

Lab. Parameters

eosinophilophil count……. Neutrophil count……. Lymphocytesocyte count……

Total serum IgE……..

Culture results :

Sputum microbiological VITEK2 diagnosis……

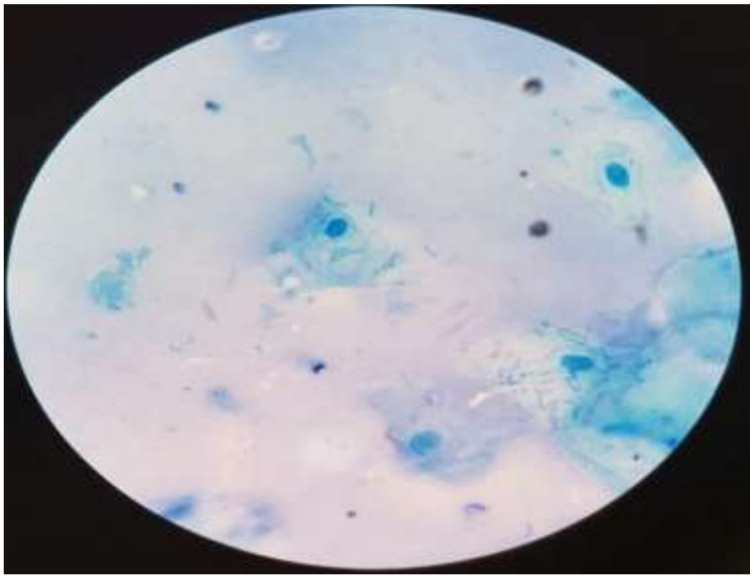
Appendix (2)VITEK2 System

Appendix (4)ELISA Model HumaReader

Appendix (5))Catalase test: *S. pneumoniaee* negative test, E. Coli positive test

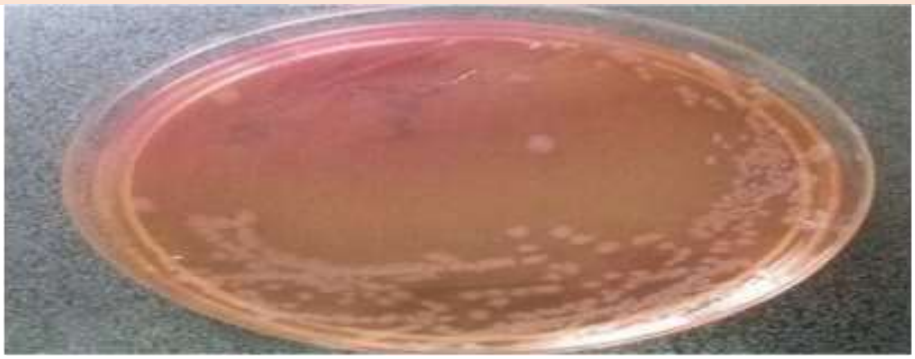
Appendix (6)Oxidase test: *S. pneumoniaee* negative test

Appendix (7)Gram stain: *S. pneumoniaee* G+ positive, diplococcic or Lancet shape



Appendix (8): Bacterial growth on MacConkey Agar.

a. *P. aeruginosa*, b. *E. coli* c. *K. pneumoniaee*

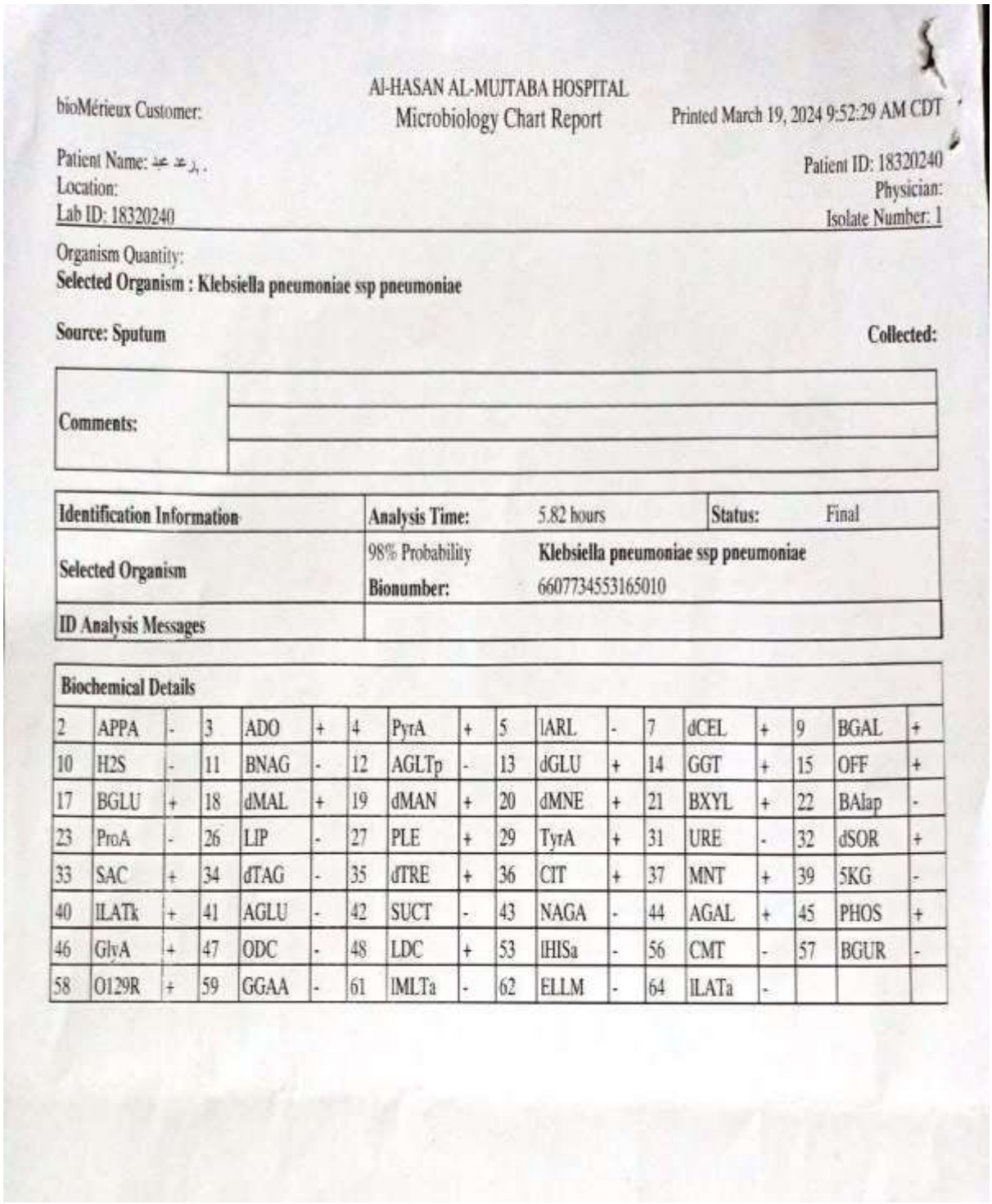


a

b

c

Appendix (9)



AL-HASAN AL-MUJTABA HOSPITAL
Microbiology Chart Report

bioMérieux Customer:

Printed March 19, 2024 9:52:29 AM CDT

Patient Name: عبد رضا .
Location:
Lab ID: 18320240

Patient ID: 18320240
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: Sputum **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:** Final
Selected Organism	98% Probability **Bionumber:**	**Klebsiella pneumoniae ssp pneumoniae** 6607734553165010	
ID Analysis Messages			

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed February 27, 2024 7:31:42 AM CST

Patient Name: 2 ,حنين احمد
Location:
Lab ID: 242202444

Patient ID: 242202444
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella oxytoca

Source: Sputum

Collected:

Comments:	

Identification Information	**Analysis Time:**	3.85 hours	**Status:**	Final
Selected Organism	98% Probability	**Klebsiella oxytoca**		
	Bionumber:	2601714476004000		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	+	39	5KG	+
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

الخلاصة

الربو هو اضطراب التهابي مزمن يصيب مجاري الهواء ويتسم بنوبات متكررة من الصفير وضيق التنفس وضيق الصدر والسعال. تؤثر التهابات الجهاز التنفسي بشكل كبير على إدارة وتشخيص الربو عند الأطفال، وغالبًا ما تؤدي إلى تفاقم الحالة وتعقيد أنظمة العلاج. تهدف هذه الدراسة إلى تقييم العلاقة بين عدوى الجهاز التنفسي البكتيرية وشدة الربو والسيطرة عليها.

هذه دراسة مقطعية حللت بيانات 100 مريض بالربو تتراوح أعمارهم بين 5 و 16 عامًا من مستشفيين في كربلاء. للفترة من أكتوبر 2023 إلى 30 مايو (2024). احتوت مجموعة العينات على عينات البلغم والسعال التي تم جمعها بعد شطف المرضى لأفواههم بالمحلول الملحي أو الماء وتم نقل العينات على الفور إلى المختبر. للتعرف على البكتيريا، يتم تطعيمها بمجموعة متنوعة من الوسائط (الهوائية) بما في ذلك أجار الصويا التربتيكازي، وأجار دم الأغنام 5٪، وأجار ماك كون كي، وأجار ملح مانيتول. ثم يتم حضانة الثقافات لمدة ليلة كاملة عند 37 درجة مئوية؛ إذا لم يُشاهد أي نمو، يتم إعادة حضانة الثقافات لمدة 24 ساعة أخرى ويتم استخدام نظام VITEK2 compact لتحديد العزلات البكتيرية.

للتحليل الإحصائي، تم تحميل البيانات في الحزمة الإحصائية (SPSS). أظهرت نتائج هذه الدراسة أن غالبية (57٪) من مرضى الربو تتراوح أعمارهم بين 5 و9 سنوات، مع ملاحظة اختلافات كبيرة (p = 0.0001). كانت الأغلبية الكبيرة من الذكور (69٪) مقارنة بالإناث (31٪) (p = 0.0001). الخصائص السريرية لمرضى الربو: كان تاريخ الإصابة بـ COVID-19 سلبيًا في 85٪. كان لدى نسبة كبيرة من المرضى (77٪) أمراض مصاحبة مثل التهاب الجيوب الأنفية والتهاب الأنف والارتجاع المعدي المريئي (p = 0.0001) حيث كان 95٪ يستخدمون الكورتيكوستيرويدات المستنشقة لأكثر من 3 سنوات.

نتائج زراعة البكتيريا: كانت *Streptococcus pneumoniae* هي الممرض الأكثر عزلاً (11%). توجد فروق كبيرة في مستويات الخلايا الليمفاوية وإجمالي IgE بناءً على نمو البكتيريا، حيث لوحظت أعلى التركيزات لدى مرضى *Staphylococcus aureus*. كما كان هناك ارتباط كبير بين ضعف السيطرة على الربو وعزل البكتيريا من *Klebsiella pneumoniae* و *Streptococcus pneumoniae* و *Pseudomonas aeruginosa*.

أظهرت نتيجة هذه الدراسة إلى أن البكتيريا سلبية الجرام أكثر بروزًا بين التهابات الجهاز التنفسي. *Streptococcus pneumoniae* هي العزلة البكتيرية الأكثر شيوعًا. كما يوجد ارتباط كبير بين ضعف السيطرة على الربو ووجود عزلات بكتيرية محددة. تتأثر العلامات المعملية مثل الخلايا الليمفاوية والغلوبولين المناعي(IgE) بنوع نمو البكتيريا، وخاصةً في المصابين بعدوى *Staphylococcus aureus*، مما قد يشير إلى استجابة مناعية لهذه الممرضات.

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

العدوى البكتيرية التنفسية التي تؤثر على الربو عند الأطفال: الارتباط بالشدة والسيطرة

رسالة

مقدمة الى مجلس كلية الطب/جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالب

مصطفى قحطان هاشم

بكالوريوس علوم حياه/ كلية العلوم /جامعة كربلاء

بأشراف

أ.د سوسن محمد جبار

أ.م.د شواق علي حسين

2024 م

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