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Ministry of Higher Education and
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University of Karbala /College of
Medicine Department of Microbiology

Respiratory Bacterial Microbiome Effects on Serum level of IL-37 and IL-38 with Correlation to the Severity of Recurrent Wheezer

A Thesis

Submitted to the Council of the College of Medicine/University of Karbala, for the partial of the Requirement for the Master Degree of Science in Medical Microbiology.

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَّرَهُ مَنَازِلَ لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابَ مَا خَلَقَ اللَّهُ ذَلِكَ إِلَّا بِالْحَقِّ يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُون 5)

صدق الله العلي العظيم

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Committee certification

We, the examiners committee, certify that we have read the M.SC. thesis entitled: **Respiratory bacterial microbiome effects on serum level of IL-37 and IL-38 with correlation to the severity of recurrent wheezer**

We have examined the student (**Dhu-ALnoorin Hassan algashami)** in it is contents. In our opinion itis meets the standards of thesis for the degree of Masters in Medical Microbiology and Immunology .

Prof. Dr.

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Head of Microbiology Department Medicine

Prof. Swasan Mohammed Jabbar ~~Mahdi~~

/ /2024

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Dedication

To

the dazzling star in my sky, to the most wonderful man I have ever known who illuminated my path. My father, leading my evenings for brighter tomorrows

.

To

my mother, the most loving person I have ever known and the source of all pure and limitless love in the universe

.

To

my friend, the person who has always supported me and held my hand through all of my endeavors:
I share all of my dreams and achievements with him. My beloved spouse

To

the faces that the illness conceals the smiles on, and to everyone who supported and assisted me in bringing this work to light...The faces that the illness conceals their smiles, and to everyone who supported and assisted me in bringing this work to light...

Dhu-ALnoorin

2024

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It gives me great pleasure to express my gratitude to everyone that supported me in finishing my thesis.

I sincerely thank each and every one of you.

summary :

According to the most recent edition of the Global Initiative for Asthma guidelines, asthma is "a heterogeneous disease, usually characterized by chronic airway inflammation. The term recurrent wheezer is commonly used now for children below 3-5 years of age. The relationship between normal flora and asthma is complex, and evidence suggests that certain commensal microorganisms may play a role in the development of asthma. Exposure to some environmental microbes during early childhood may play a crucial role in promoting normal immune system development and preventing asthma. The processes leading to the development of asthma are greatly influenced by the interaction between the host's immune system and microbiota.

A case control study was conducted for a period of 6 months, starting from August /2023 to January/2024; the total number of participants were 100 subjects; they were divided into two groups: the first one includes patients with pediatric asthma and \or recurrent wheezer while the second group includes healthy control. Laboratory tests were done by serological techniques (sandwich ELISA), and samples were tested for specific serum human IL-37, IL-38, and total IgE. Swabs were taken from all participants and performed for bacteriological examination profile (culturing morphologically, microscopically, and confirmative test by VITEK 2 compact system).

Significant differences appeared in mean serum levels of IL-37 and IL-38 within the studied groups, both markers were significantly increased in the patient group . in addition to all blood parameter (lymphocyte , eosinophil , neutrophil count) have highly significant levels within patient groups. IL-

37 and IL-38 were shown by ROC curve analysis to be able to distinguish and diagnose patients with recurrent wheezers from healthy controls. It might therefore be considered useful biomarkers for diagnosis.

Culturing investigation depending on morphological and VITEK compact system results showed that the patient group reveals bacterial growth as follow: Staphyloococcus. aureus12 (24.00) % , then Streptococcus. Pneumonia 11(22.00) % . Followed by, Haemophilus 6 (12.00) % and K. rosia 6 (12.00) %, Moraxella 5 (10.00) % , Staphylococcus. epidermidis 4(8.00) %, Streptococcus. pyogen 3(6.00) % and , lastly, Pseudomona 1(2.00) % and S. xylose 1(2.00) % . Whereas, control group recorded that Staphylococcus. saprophyticus was most isolated bacteria in this study with percentage of 12(25.50) % followed by Streptococcus pyogen 10(19.60) % then Staphylococcus epidermidis 10(19.60) % and Streptococcus mutans 7(13.70) % and Haemophilus 5(9.80) % and Staphylococcus aureus 3(5.90) % lastly Kocuria rosea 2(3.90) % and Corynebacterium 1(2.00) %.

For conclusion, There is a marked elevation of levels of both IL37 & IL38 in recurrent wheezers which may serve as inflammatory markers or a precursor to the disease process and could be considered good diagnostic biomarkers for recurrent wheezers. The combination of Staph. aureus and Strep. pneumoniae was higher in asthmatic patients, while coagulase-negative staphylococci were considerably higher in healthy controls. Microbial dysbiosis could promote an unbalanced immune response and contribute to the development of allergy disorders, particularly asthma.

List of Contents

List of Tables

List of figures

List of abbreviation

Code	word
aBA	**Allergic bronchial asthma**
AD	**allergic diseases**
AHR	**Allergic hyperresponsiveness**
AIT	**Allergy immunotherapy**
APCs	**Antigen presenting cells**
AR	**allergic rhinitis**
AUC	**area under the curve**
BA	**Bronchial asthma**
BHI	**Brain heart infusion**
BHR	**bronchial hyperresponsiveness**
CBC	**Complete blood count**
CI	**confidence interval**
CLR	**C-type lectin receptors**
CLRs	**lectin recep- tors**

CONS	**Coagulase negative staphylococci**
COPD	**Chronic obstructive pulmonary disease**
CS	**Caesarian section**
DC	**dendritic cell**
EAACI	**European Academy of Allergy and Clinical Immunology**
(EIA).	**enzyme immunoassay**
(ELISA).	**Enzyme-Linked Immunosorbent Assay**
EBC	**eosinophil blood count**
ECs	***Epithelial-derived cytokines***
ED	**emergency department**
EDN	**eosinophil-derived neurotoxin**
ERK	**extracellular signal-regulated kinase**
FEV1	**Forced vital capacity**
FVC	**Forced vital capacity**
GINA	**Global Initiative**
GM	**gut microbiota**
GM- CSF	**granulocyte-macrophage colony-stimulating factor**
GWAS	**genome-wide association studies**
HKIA	**Hong Kong Institute of Allergy**
ICS	**inhaled cortecosteroid**
IFN	**Interferon**
IgE	**Immunoglobulin E**
IgE	**Immunoglobulin E**

IgG	**Immunoglobulin g**
IL	**Interleukin**
IL-1R1	**interleukin-1 receptor 1**
IL-36R	**interleukin-36 receptor**
IL-37	**Interleukines 37**
IL-38	**Interleukines 38**
ILC2	**innate lymphoid cells**
ILs	**Interleukins**
JNK	**Janus Kinase**
K. rosea	**Kucoria rosia**
LMICs	**Low-Middle Income Countries**
LPS	**lipopolysaccharides**
MBP	**major binding protein**
MRSA	**Methesiline resistance staphylococcus aureus**
MSA	**Mannitol Salt Agar**
N	**number**
NFA	**near-fatal asthma**
NK	**natural killer**
NLRs	**Nod-like receptors**
NP	**nasopharngeal**
NPV	**negative predictive value**
OD	**optical density**
OPS	**Oropharngeal swab**
PA	**Pediatric Asthma**
PBMCs	**peripheral blood mononuclear cells**

PPV	**Positive predictive value**
RIG-I	**retinoic acid-inducible Gene-I**
ROC	**Receiver Operating Characteristics**
RSV	**Respiratory syncytial virus**
RTIs	**Respiratory tract infection**
RV	**rhinovirus**
SES	**socioeconomic status**
Std	**Stander deviation**
TH	**T-helper cells**
TLR	**Toll-like Receptors**
TNF	**tumour necrosis factor**
Tregs	**Regulatory T cells**
TSLP	**thymic stromal lymphopoietin**
URT	**Upper respiratory tract**

Chapter one

Introduction And literature review

1.1 Introduction:

Asthma is the most prevalent chronic illness in children and a constant strain on the medical system. A growing number of children and adolescents worldwide have been exhibiting symptoms of asthma in recent years, with Low-Middle Income Countries (LMICs) having the highest frequency. This trend seems to be influenced by host variables (genetics, atopy) as well as environmental factors (microbial contamination, exposure to passive smoking, and air pollution) (Ferrante *et al.*, 2018).

The Global Initiative for Asthma (GINA) definition of asthma was recently updated; asthma is proposed to be ‘‘a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation’’(Papadopoulos *et al.*, 2019)

The microbiota of the respiratory tract probably acts as a gatekeeper that provides resistance to colonization by respiratory pathogens. The respiratory microbiota might also be involved in the maturation and maintenance of homeostasis of respiratory physiology and immunity (Man *et al.*, 2017) . In addition to being influenced by outside variables like respiratory virus illnesses, the baby airway microbiota may have predictive value for the eventual development of asthma in childhood. The clinical course of asthma, including exacerbation rates and control degree, has also been linked to the airway microbiota (Rhia *et al.*, 2021).

Interleukins (ILs) are signaling molecules that are crucial in regulating immune responses during infectious diseases. Pro-inflammatory ILs contribute to the activation and recruitment of immune cells, whereas anti-inflammatory ILs help to suppress excessive inflammation and promote tissue repair(Al-Qahtani *et al.*, 2024)

Interleukin (IL)-38 it is a pro-inflammatory cytokine recently been reported to exert an anti-inflammatory function by binding to several receptors, including interleukin-36 receptor (IL-36R), interleukin-1 receptor accessory protein-like 1 (IL-1RAPL1), and interleukin-1 receptor 1 (IL-1R1) to block binding with other pro-inflammatory cytokines and inhibit subsequent signaling pathways; thereby regulating the differentiation and function of T cells, peripheral blood mononuclear cells, macrophages, and dendritic cells. Recent findings have shown that abnormal expression of IL-38 in inflammatory autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, primary Sjogren's syndrome, psoriasis, inflammatory bowel disease, hidradenitis suppurativa, ankylosing spondylitis, and glaucoma, involves Th1s, Th17s, and Tregs(Xie *et al.*, 2019).

IL-37 It is a brand-new immunomodulatory cytokine that reduces inflammation. In particular, it lessens the generation of cytokines that are anti-inflammatory, which in turn suppresses immunological and inflammatory reactions. According to (Zeng *al.*, 2022), IL-37 has three main functions: it inhibits kinase signaling activation, lowers transcriptional cytokine expression, and decreases the synthesis of pro-inflammatory cytokines.

Aim of the study

the goal of the study is to evaluate the association between the bacterial respiratory microbiome, IL-37, and IL-38 with asthma severity, type, and control through the following objectives:

1. Identify the type of bacterial microbiome in the respiratory tract of children with asthma and in healthy control children.
2. ELISA detection of serum total IgE IL-37 and IL-38 in both patients and control groups.
3. Correlate specific bacterial microbiota growth & serum IL-37 and IL-38 with laboratory blood tests as CBC (eosinophil ,neutrophil ,lymphocytes).
4. Correlate specific bacterial microbiota growth and serum IL-37 and IL-38 with asthma severity, type, and control.

There for identification of which microbiome growth is associated with more severe or uncontrolled asthma or allergic asthma is essential for a better understanding of the pathophysiology of pediatric asthma in Iraq. in addition to recognition of IL-37 and IL-38 role in the pathogenesis of asthma.

1.2. Literature Review

1.2.1. Definition of Pediatric asthma:

Pediatric asthma is the most common chronic childhood disease in the USA, currently affecting ~7 million children. This heterogeneous syndrome is thought to encompass various disease phenotypes of clinically observable characteristics (Authelet- *et al.,* 2018) .

Asthma is a chronic inflammatory disease, with two main endotypes: Type 2-high and Type 2-low inflammation. Type 2-high inflammation is driven by Type 2 activation, leading to production of IL-4, IL-5 and IL-13 in response to varying stimuli (e.g., allergens, viral infection). These cytokines activate and recruit airway eosinophils, stimulate IgE production, raise exhaled nitric oxide (feno) and ultimately lead to airway remodelling (Canonica *et al.,* 2021).

Asthma is an obstructive lung disease affecting >230 million people worldwide and a significant cause of morbidity in patient of all ages. It is a heterogeneous disease with a complex pathophysiology And phenotype . Understanding the severity of the disease is important, and treatment is aimed at symptom control And the prevention of future exacerbations . However, understanding an individual's disease phenotype, endotype ، And comorbidities is necessary for asthma treatment, with appropriate consultation with asthma Specialists required for those with severe asthma **(**SO *et al.,* 2018).

Impacting Pattern-Recognizing Receptors (PRRs) of innate immunity can modify allergic responses. Toll-like Receptors (TLR), Nod-like receptors (NLRs), C-type lectin receptors (CLR), retinoic acid-inducible Gene-I (RIG-I)-like receptors (RLRs), and nucleotide-binding domain receptors

are the most studied PRRs (Muñoz-Wolf et al., 2016). Patients with asthma who also have reflux disease, allergic rhinitis, obesity, depression, diabetes mellitus, and cardiovascular diseases, particularly those who have severe asthma (Rogliani *et al.,* 2022) .

1.2.2. The Definition Of Microbiota That Participate In Asthma

The human microbiome is a complex and highly diversified population of microorganisms that inhabits every part of the body, including the mouth cavity, skin, gastrointestinal tract, esophagus, and lung. These microorganisms also include their genes and metabolic products. Numerous genetic, dietary, and environmental factors—such as age, mode of birth, and antibiotic use—shape and alter the microbiome (De Vos *et al.*, 2022).

All of the bacteria (the group of microbial genomes) that live on or in human tissues and biofluids together make up the human microbiome. Human microbiomes are primarily found in the stomach, but they can also be found on the skin, in mammary glands, seminal fluid, the uterus, ovarian follicles, the lung, saliva, the oral mucosa, the conjunctiva, and the biliary tract (Marchesi *et al.,* 2015) .

The microbiota of the respiratory tract probably acts as a gatekeeper that provides resistance to colonization by respiratory pathogens. The respiratory microbiota might also be involved in the maturation and maintenance of homeostasis of respiratory physiology and immunity (Man *et al.,* 2017). The development of asthma in later childhood may be predicted by the baby airway microbiome, which may also be impacted by exogenous factors such respiratory virus infection. Additionally linked to the clinical course of asthma, including exacerbation rates and control levels, is the airway microbiota (Rhia *et al.,* 2021) .

Certain studies indicate that interaction with maternal microorganisms is the source of the early colonizers of the baby's gut (Palmer *et al.*, 2007) . Compared to babies delivered vaginally, newborns delivered by cesarean section have a distinct microbiota (Dominguez-Bello *et al.*, 2016) **.** The following are also crucial for fostering a healthy microbiome in newborns: delivery at full term, nursing, and encounters with diverse microbes (Mohajeri et al., 2018)(Gritz *et al.*, 2015). As early as six months of age, the introduction of solid food causes the microbiome to begin to become more like an adult (Mohajeri *et al.*, 2018) .

1.2.2.1 The respiratory microbiome (nasopharngeal) in pediatric asthma .

Moraxella was more enriched in the NP of patients with a history of up to three RTIs (esposito, Susanna, *et al.*, 2022) . *Prevotella, Streptococcus,* and *Veillonella* are the most prevalent genera in the respiratory system, whereas *Firmicutes* and *Bacteroidetes* are the most prevalent phyla (Charlson *et al.*, 2011) . The nasopharyngeal microbiota makeup of a cohort of Egyptian children with asthma differed markedly from that of their counterparts in good health. While the combination of *Staph.aureus* and *Strep. pneumoniae* was higher in asthmatic patients, coagulase negative staphylococci (CoNS) were considerably higher in healthy controls. It was discovered that children with more severe asthma and younger asthmatics were more likely to have *Kocuria rosea* (basanti, Christine WS, *et al.*, 2021) .

1.2.2.2 Percentage of microorganisms in asthmatic patients

Percentage of microorganisms by Gram's staining in asthmatics, chronic bronchitis, and controls cases were 93.33%, 86.67%, 76.67% respectively. Among the pathogenic bacteria, percentage of significant count of *Staphylococcus aureus* and *Klebsiella pneumonia* were found to be high in

chronic bronchitis cases when compared these pathogens in asthmatics. In bronchial asthma cases, the percentage of significant count of *Streptococcus pyogenes, Proteus mirablis and Pseudomonas aeruginosa* were high as compared to chronic bronchitis cases. In chronic bronchitis patients and asthmatics, significant count of non-pathogens were 16.67% and 13.33% respectively (Tomar *et al.,* 2020) .

There was a significant statistical difference (p=0.007) in the total microbiome makeup between children with asthma and control children. *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most prevalent combination of organisms in asthmatics, and their frequency was statistically greater (0.038) than that of controls. Compared to the asthmatic group (3.3%), normal controls had a greater incidence of coagulase negative *staphylococci* (30%) (p-0.009). Even though asthmatics had higher levels of *Kocuria rosea*, the difference was not statistically significant. On the other hand, patients with more severe asthma were found to have more *K. rosea* in their group (p=0.02) (Basanti *et al.,* 2021).

It has been suggested that the exposure to some microorganisms or microbial-derived components administered orally might influence the development and functions of gut and, secondarily, airway microbiota, mimicking the protective natural exposure that is needed for a healthy respiratory system (Esposito *et al.,* 2018)(Esposito *et al.,* 2022) .

1.2.3. Definition Recurrent Wheezers.

Recurrent wheezing, a common diagnosis after severe bronchiolitis, has multiple phenotypes of uncertain relation to childhood asthma. Depending on the phenotype, 33% to 54% will develop asthma by age 6 years (mansbach, Jonathan M., *et al.* 2023) . Young children with persistent

wheeze are at risk for childhood asthma. Thus, identification of risk factors associated with persistent wheeze in young children with recurrent wheeze might aid in early detection of asthma and initiation of preventative therapies (hardee, Isabel J, *et al.*, 2024).

in review introduce by the European Academy of Allergy and Clinical Immunology (EAACI) Preschool Wheeze Task Force aimed to provide systematic evidence for the association between increased EBC and the risk of future asthma, That study data demonstrated that high EBC in preschool children with wheezing is associated with future asthma development (adamiec, Aleksander, *et al.*, 2024) .

1.2.4. Epidemiology of Asthma.

In united States asthma was more prevalent among boys (9.2%) than among girls (7.4%), children aged ≥5 years (approximately 10%) than children aged <5 years (3.8%) (zahran et al., 2018). The adjusted prevalence of asthma ranged from 4.4% [95% CI: 4.0–4.8%] in Turkey(Tarraf *et al.*, 2018). The prevalence of current symptoms of asthma in the Syria (wheezing in the last 12 months) in different centres ranged from 4.7% to 5.7% for 6-7-year-olds and 3.9% to 6.5% for 13-14-year-olds(Mohammad *et al.*, 2010) . the overall prevalence of asthma in children from Saudi Arabia has been reported to range from 8% to 25%, based on studies conducted over the past three decades(Al-Moamary *et al.*, 2019)

In a study was conducted in Saudi Arabia within 2016,the prevalence of wheezing in12 months was 18.2% and the difference between men and women was not significant(Al Ghobain *et al.*, 2018). The prevalence asthma in Jordan was Primary schoolchildren aged 6-7 years had significant wheezing ever (27.2%)and older children (25.1%; p <

0.05)(Abu-Ekteish *et al.,* 2009) . Prevalence of asthma in Iraq's neighbors such as the prevalence of asthma in Kuwait was estimated to be 15% of adults and 18% of children (93,923 adults; 70,158 children)(Khadadah, 2013) . In Tikrit the prevalence of asthma when assessed by interview was 7.2% [95% confidence interval (CI) 6.3% - 8.3%], while it was 8.9% [95% CI 7.9 % - 10.0%] when assessed by clinical examination(Alsamarai *et al.,* 2009) .

1.2.5. Risk Factor For Preschool Wheezing And Progression To Childhood .

1.2.5.1 -Smoking

The mechanisms of smoking related lung disease can include increased small-airway inflammation with neutrophils and macrophages, airway hyper-responsiveness, and airflow obstruction (McCrea KA *et al.,* 1994) (Hancox *et al.,* 2016) . Smoking is an independent risk factor for the development of asthma symptoms (Rasmussen *et al.,* 2000).Passive smoking or environmental tobacco smoke consists of the direct inhalation of tobacco smoke (secondhand smoke) as well as exposure to tobacco residue left behind after smoking (third-hand smoke). Although active smoking and secondhand smoking have been well recognized to increase the risk of exacerbations, there is increasing evidence that third-hand smoking is also associated with increased lower respiratory symptoms (Jung JW *et al.,* 2012).

1.2.5.2-Obesity

Obese youth are not only more likely to develop asthma, but they may be more likely to have severe asthma, resulting in a greater need for health care utilization and aggressive asthma treatment (Black *et al.,* 2013). Obesity is very common in severe asthmatics. At least two distinct obese

asthma phenotypes have been characterized, one an early onset, allergic phenotype and the other a late onset, female dominated, non allergic phenotype(Tashiro and Shore, 2019)

1.2.5.3-Socioeconomically status

Low socioeconomic status (SES) has been linked to higher morbidity in patients with chronic diseases, but may be particularly relevant to asthma, as asthmatics of lower SES may have higher exposures to indoor (e.g., cockroaches, tobacco smoke) and outdoor (e.g., urban pollution) allergens, thus increasing risk for exacerbations(Bacon *et al.*, 2009) . Asthma and asthma-related symptoms occurred more frequently in urban than in rural areas, and that difference correlated with environmental risk exposures(Jie *et al.*, 2013) . Asthma incidence and morbidity are greater in urban areas (Gern, 2010). Environmental air pollutants increased the risk of asthma among children . Exposure to congested roads, lorry fumes, and indoor carpet were associated with asthma among children (Idris *et al.*, 2016).

In recent study , Compared with children of higher socioeconomic status (SES), those in the lowest SES group had increased risk of asthma exacerbation, hospital admission, and receiving treatment for severe asthma symptoms (Lee, W. S, 2023).

1.2.5.4-Genetic-Environmental Risk Factors Of Asthma.

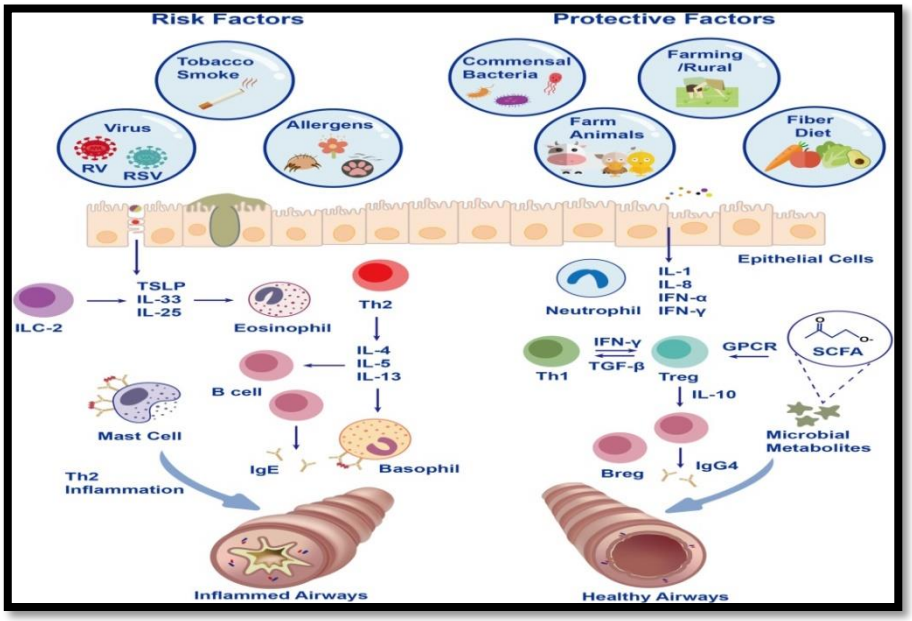
Asthma is a complex disease, and its incidence is determined by an intricate interplay of genetic and environmental factors. The identification of novel genes for asthma suggests that many genes with small effects rather than few genes with strong effects contribute to the development of asthma(von Mutius, 2009) . The prevalence of asthma has increased rapidly when only changes in exposure to environmental factors; which go

together with changes in lifestyle, are likely to explain such a rapid increase. Exposure to allergens is a risk factor for allergic sensitization, and allergic sensitization is a risk factor for allergic asthma(Leynaert *et al.*, 2019) . Environmental exposures interplay with human host factors to promote the development and progression of allergic diseases, the environmental changes result in increased exposure to air and traffic pollution, fungi, infectious agents, tobacco smoke, and other early-life and lifelong risk factors for the development and exacerbation of asthma and allergic diseases(Murrison *et al.,* 2019).

Recent genome –wide association studies (GWAS) reported that the identified genetic variants only explained approximately 9% of the asthma heritability (Han *et al.*, 2020) . Such "missing" heritability in GWAS studies could be attributed to increased penetrance of asthma susceptibility genotypes by environmental factors that manifest their effects through gene–environment interactions(Kabesch *et al.*, 2020) . Genetic association studies have allowed identifying the 17q21 locus and the *ADRB2* gene as the loci most consistently associated with asthma exacerbations(Herrera-Luis *et al.,* 2019).

Genetic variations in different loci and genes are important in asthma pathogenesis. There is much importance of various immunological pathways in the IgE secretion regulation. Alterations in any main part of these pathways can increase the risk of asthma development . Polymorphisms in these genetic markers can effect certain pathways which predict the asthma susceptibility(Saba *et al.,* 2018). The risk of personal asthma increased in relation to both parents' asthma and siblings' asthma, and showed a dose-response pattern with increasing number of siblings having asthma. The incidence rates of asthma were the highest among those who had more than one first-degree relative with asthma, including

parents and siblings (Paaso *et al.*, 2013) . Family history of asthma in grandparent may be a significant risk factor for asthma in granddaughters' compared to grandsons. Thus genetics of asthma may skip generations(Sheikh *et al.*, 2013) .



Figure(1.1): Environmental Factors Impacting Preschool Wheezing And Asthma (Sheikh *et al.*, 2013) .

1.2.5.5-Respiratory Virusis

Between 10% and 30% of preschoolers experience early wheezing, or bronchiolitis, which can occur before an asthma diagnosis is made (Jartti *et al.*, 2019).

Respiratory viral infections are often the cause of wheezing disorders in children, understanding the viral etiology may help to understand how symptoms arise and the condition progresses. The primary cause of bronchiolitis in infants is the respiratory syncytial virus (RSV), while rhinovirus (RV) is the main cause of wheezing in children after that (Jartti *et al.*, 2020) Compared to young children who suffer wheezing generated

by other viruses, those who wheeze with RV are more likely to develop recurrent wheezing and subsequently asthma (Makrinioti *et al.*, 2022)

The synergy between rhinovirus (RV) infection and Th2 responses is mediated by two important components, which have been identified: reduced antiviral responses and increased Th2 inflammation induce by RV (Jackson *et al.*, 2022) . A later study, on the other hand, revealed that infants infected with RSV but not RV had elevated Th2 and Th17 pathways and downregulated interferon (IFN) responses, which were linked to recurrent wheezing in the ensuing two years (Turi KN *et al.*, 2018) . accompanied with a thorough discussion of the molecular targets and the possible connection between the respiratory microbiome and the etiology of respiratory diseases. Currently, the primary obstacle to not considering the respiratory microbiome as a novel druggable target for therapeutic intervention is the lack of strong evidence supporting the association between the microbiome and illness etiology (Alsayed *et al.*, 2023) .

1.2.6. Pathogenesis of Asthma

The capacity of immune system cells to produce inflammatory cytokines and establish a memory response to microbes is already evident in the foetal gut from the second semester of pregnancy, being partially dependent on the maternal microbiome and relevant for the development of asthma (Mishra *et al.*, 2021)

A variety of immune cells, such as mast cells, eosinophils, neutrophils, T lymphocytes, and airway epithelial cells, all play a role in the hyperresponsiveness and inflammation of the airways during the course of asthma development, which results in a variable and broad restriction of expiratory airflow. Asthma pathophysiology has been shown to entail

dysregulation of both innate and adaptive immune responses as a result of increased oxidative stress and abnormal progressions of glycolysis, fatty acid metabolism, and amino acid metabolism in various immune cells (Qin *et al.,* 2024)**.**

One of the main signs and symptoms of asthma in both adults and children is reversible airflow limitation. The heterogeneous nature of asthma is associated with a number of risk factors, including as pollution, smoking, obesity, microbes, and family history. This helps to explain the variety of asthma phenotypes that present in various clinical and pathological features (Beasley, Semprini, and Mitchell 2015). The following conditions may cause clinical symptoms: airway inflammation, airway blockage, or airway hyperresponsiveness (AHR). These conditions include coughing, dyspnea, chest tightness, and wheezing. Moreover, the immunological response brought on by genetic and environmental factors, which includes altered cellular function and disturbed metabolic processes, is a major player in the

pathophysiology of asthma (Papi *et al.,* 2018) . Airway inflammation in asthma is caused by the recruitment of mast cells, eosinophils, B cells that produce IgE, and IgE that is dependent on T helper type 2 (Th2) cells. (Holgate, 2012) . In addition to allergen-specific Th2-driven adaptive immunity, innate immune cells such as mast cells, eosinophils, granulocytes, epithelial cells, type 2 innate lymphoid cells (ILC2), dendritic cells (DCs), natural killer (NK) cells, and others are believed to be involved in both allergic and non-allergic pathogenesis mechanisms in asthma extracellular acidification rate (Chatila ,2016) .

Asthma flare-ups are believed to be closely linked to immune cell metabolism during pathogenic conditions (Lancet 2015). Basic cellular metabolic processes and disease-related immune responses have been

directly linked to asthma etiology (Holgate 2012 and Ayres 2020). Knowing the metabolic process of immune cells linked to asthma and how it impacts immunologic activities and functions might help explain the role of immunometabolism in the etiology of asthma. Moreover, the primary proteins in associated signaling cascades may offer new targets for future therapeutic approaches (Qin *et al.*, 2024).

Intricate immunological, genetic, and environmental factors all play a role in the pathophysiology of asthma. An ongoing inflammatory response of the airway wall, mediated by a variety of immune cells such as mast cells, eosinophils, dendritic cells, neutrophils, lymphocytes, and innate lymphoid cells, is what causes obstruction of the airway. Both neutrophilic and eosinophilic asthma are recognized as distinct types of the disease. Essential features of eosinophilic asthma include eosinophilia, mucus hypersecretion, bronchial hyperresponsiveness, and IgE production. Th2 cells or ILC2s that produce cytokines such as IL-4, IL-5, and IL-13 mediate inflammation in eosinophilic asthma. Asthma characterized by neutrophil infiltration is a variant of Th1 and Th17 cells, which release IL-17 and interferon gamma. (Gans *et al.*, 2020) (Hammad *et al.*, 2021) (Jeong *et al.*, 2021).

1.2.6.1 The Immune Cell Is Intrinsically Involved In The Pathogenesis Of Asthma.

Th2 cells mediate allergic inflammation via generation of a host of cytokines including IL-4, IL-5, IL-9, and IL-13 which contribute to Immunoglobulin E (IgE) production, mast cell degranulation, eosinophil recruitment, and release of inflammatory mediators such as histamine and leukotrienes (Lambrecht *et al.*, 2015). An imbalance between TH1 and TH2 pathways has been implicated in the pathophysiology of the disease

with the disease further dichotomised according to whether TH2 levels are high or low (Fahy *et al.*, 2015) . Antigen presenting cells (APCs) present inhaled allergens to T-helper cells (TH) which activate the humoural immune system via cascades of interleukins (IL). Two subclasses of TH cells are implicated in airway inflammation TH1 and TH2 ,TH1 cells produce mainly IL-2 and interferon gamma and are crucial in orchestrating many cellular defence mechanisms against infection, often mediated by neutrophilic inflammation (Berger *et al.*, 2000).

1.2.6.2 Role Of Eosinophils In Asthma Pathogenesis.

Eosinophils' function in the etiology of asthma It has pro-inflammatory and immunomodulatory effects. Eosinophils from the bloodstream are drawn to the lung in type 2-high asthma, where Th2 cell-released IL-5 activates them. Many different mechanisms may be used by activated eosinophils to carry out their biological effects in the lung, such as cytotoxic proteins

(e.g., leukotriene C4, IL-5, IL-9, IL-13, and eosinophil-derived neurotoxin (EDN), Th2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-25), acute proinflammatory cytokines (e.g., tumor necrosis factor (TNF)-a, IL-1b, IL-6, and IL-8), chemokines, and lipid mediators, such as leukotriene C4. A number of characteristics of asthma, such as BHR and goblet cell metaplasia, are influenced by all of these variables. Furthermore, the production of cytotoxic granule proteins (MBP, EPO, and EDN) caused by persistent airway inflammation linked to eosinophils damages lung structural cells (Can˜as *et al.*, 2018) **.** Thus, smooth muscle thickening, goblet cell metaplasia, and extracellular matrix protein deposition are the hallmarks of airway remodeling caused by eosinophil-associated fibrogenic factors (e.g., TGF-b) (Kanda *et al.*, 2020). Additionally, the activation of eosinophils and their release of granule material in the vicinity of airway

neurons can alter the tone of parasympathetic and sensory nerves, hence boosting beta heart rate (Drake *et al.*, 2018).

1.2.6.3 Epithelial – derived cytokines contribute to type 2 –high asthma.

Type 2-high asthma is influenced by cytokines generated from epithelial cells. Numerous pattern recognition receptors, including toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), protease-activated receptors, and purinergic receptors, are expressed by lung epithelial cells (ECs). These receptors enable ECs to produce chemokines and cytokines in response to a range of external stimuli (Figure 2) (Willart *et al.*, 2012).

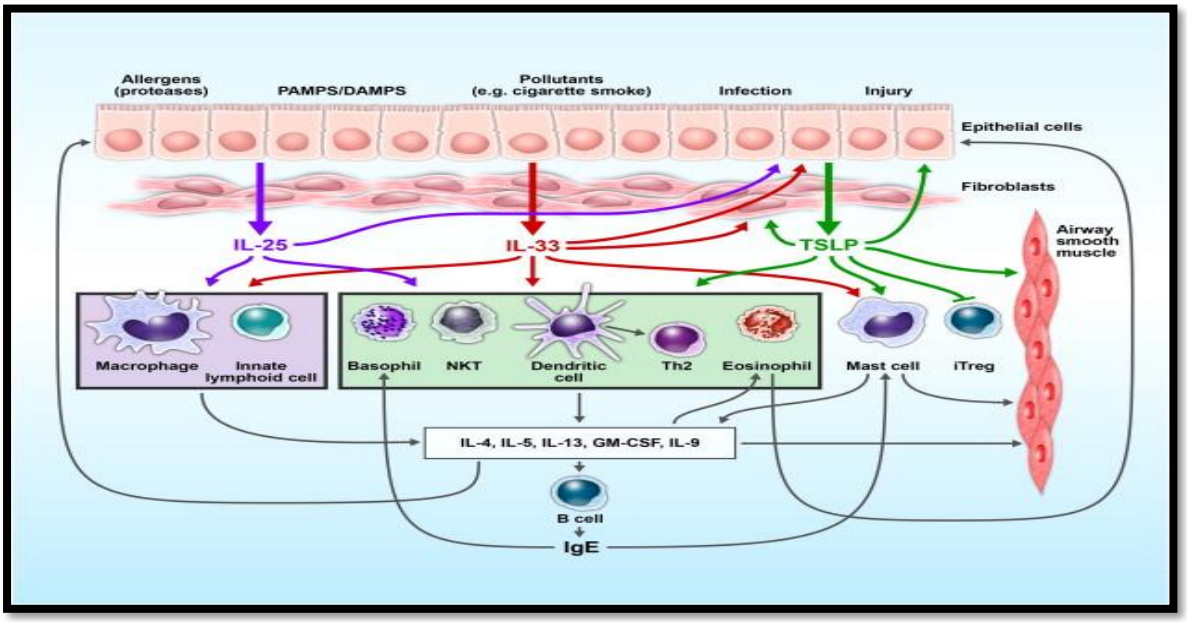


Figure (1.2.) : Dynamic role of epithelium-derived cytokines in asthma – ScienceDirect **(Willart *et al.*, 2012)**.

1.2.6.4. Contribution Of B Cell And Ige To Asthma .

A portion of the Th2 cells produced in the lymph nodes that drain the lungs will interact locally with B cells, which develop into plasma cells and cells that produce antibodies. The Th2 cytokines IL-4 and IL-13 cause B cells to generate IgE in a preferred manner. There is evidence that IgE production can occur in the lung mucosa of asthma patients, despite the fact that it primarily occurs in secondary lymphoid organs (so-called local IgE production) (Manise *et al.*, 2013).

IgE plays a multifaceted role in allergic asthma, as evidenced by its capacity to impact many immunological and structural cells. Both the low-affinity CD23 receptors and the high-affinity FcεRI can bind to IgE. FcεRI is expressed on airway smooth muscle cells, endothelial cells, epithelial cells, mast cells, eosinophils, and DCs (Redhu and Gounni, 2013).

IgE is mostly produced in asthma from a pool of memory IgG-positive B cells that class switch to IgE and develop into long-lived plasma cells in response to TFH cell-derived IL-4 and/or IL-13 (Hoof *et al.*, 2020).

1.2.7 Cytokines and Chemokines in allergic diseases.

Cytokines and chemokines are a rapidly expanding research field in laboratory medicine, inflammation and allergic disease. Inflammatory diseases are generally associated with dysregulation of cytokines and chemokines such as the imbalance of T helper (Th) type 1 and 2 cytokines, over-activation of Th17 cells, aberrant regulatory T/B cells and regulatory cytokines, and cytokine/chemokine storm. This leads to the inflammatory reaction, including infiltration of immune cells (e.g. T cells, macrophages, dendritic cells, eosinophils, basophils, type 2 innate lymphoid cells) into the inflammatory sites, increased intercellular interaction, tissue/cell damage, airway remodeling, mucus secretion in allergic disease. Therefore,

the evaluation of the immunopathological roles and dysregulation of cytokines and chemokines in inflammatory diseases will not only further foster our understanding of the immunopathological mechanisms but also furnish a biochemical basis for the development of disease activity markers and novel therapeutic cytokine-targeting agents in allergic diseases (wong 2023).

1.2.7.1 Produce and Synthesis of IL-37.

Numerous cell types, including epithelial cells, keratinocytes, tubular epithelial cells, and immune cells like activated B cells, plasma cells, macrophages, natural killer cells (NKs), dendritic cells (DCs), and CD4+ regulatory T cells (Tregs), have been shown to produce IL-37 in the cytoplasm or nucleus (Fonseca-Camarillo et al., 2015)(Shuai *et al.*, 2015)(Rudloff *et al.*, 2017).

When IL-37 is in a pathological state, its production and secretion patterns change. When immune cells and peripheral circulation are exposed to inflammatory stimuli like lipopolysaccharides (LPS) (Jia *et al.*, 2018), Toll-like receptor (TLR) agonists, and cytokines like transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), IL-1β, or IL-18 (Boersma *et al.*, 2021)(Wu *et al.*, 2021). Currently, it is believed that IL-37 expression only rises in highly inflammatory states in order to prevent immunological storms and inflammatory intensification, not in non-inflammatory or mildly inflammatory settings (Jia *et al.*, 2018).

1.2.7.1.1 .The Site and structure of IL-37 .

Interleukin-37 (IL-37) is a member of the IL-1 family, along with IL-1α, IL-1β, IL1Rα, IL-18, IL-36α, IL-36Rα, IL-36β, IL-36γ, IL-38, and IL-33. In 2000, IL-37 was identified through the independent description of five

mRNA transcripts of this cytokine by three research groups utilizing in silico technologies(Ménard *et al.*, 2000)(Yarden *et al.*, 2001) (Bery *et al.*, 2019) **.** The majority of the IL-1 family cytokine genes are situated on chromosome 2q12–13, which is where IL-37 is found (Santin *et al.*, 2008)**.** The exons 4, 5, and 6 are present in the isoforms a, b, and d. These exons seem to be connected to the biological roles of IL-37 (Junttila *et al.*, 2011) **.**

1.2.7.1.2 IL-37 Receptor Complex And Signaling Pathways.

The IL-37 receptor complex is similar to the IL-18 receptor, another representative of the IL-1 family. IL-18 is one of the key pro-inflammatory cytokines acting as a pathogenetic factor in a number of diseases (Deyev *et al.*, 2017)(Proshkina *et al.*, 2019) .

IL-37 binding and signal transduction. IL-37 binds to IL-18Rα instead of IL-18 and recruits the orphan decoy IL-1R8, which leads to suppression of innate and acquired immunity, as well as augmentation of anti-inflammatory pathway, instead of activation of the IL-18 pathway. Adapted from Novick and Dinarello (Novick *et al.*, 2013)(Dinarello *et al.*, 2016) . **as figure (1.3)**

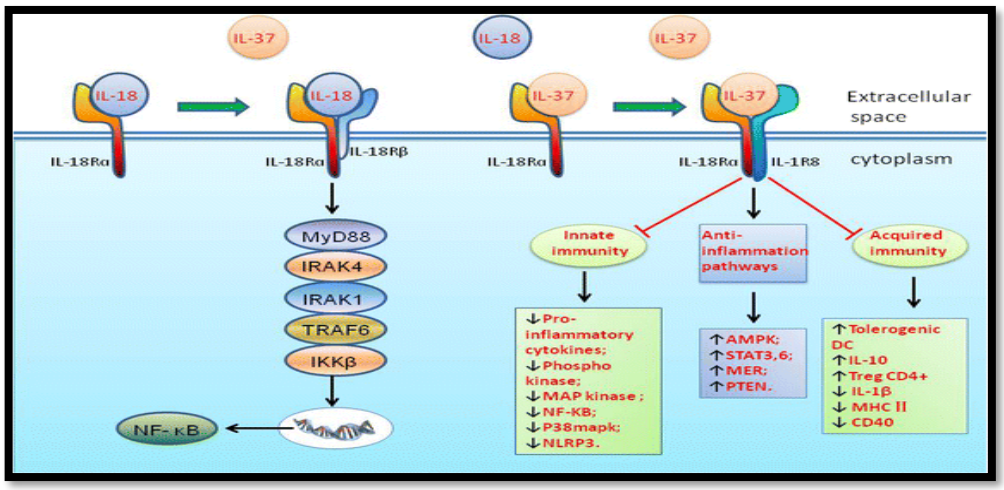


figure (1.3): IL-37 binding and signal transduction (Dinarello et al., 2016).

1.2.7.1.3 . Characteristic and Importance Increased of IL-37.

In contrast to the majority of IL-1 family members, IL-37 functions as a negative regulator of inflammation. Inflammatory cytokines and chemokines are suppressed when IL-37 is activated, which in turn stops pro-inflammatory cells—primarily neutrophils and eosinophils—from invading the area. It is unclear exactly what cellular and molecular processes underlie IL-37's anti-inflammatory effects in the emergence of allergy disorders (AD) (Shilovskiy *et al.,* 2019) .

The strong IL-37 expression in plasma cells (both in normal and in pathological tis- sues), as well as B lymphoma cells, suggests a potential role in immunoglobulin production and B cell activation in diseases such as multiple myeloma and B cell lymphomas, colon carcinoma, and inflammatory bowel disease (Kumar *et al.,* 2002).

Ten years after the first description of IL-37, it is becoming evidence that this cytokine may set a new paradigm for regulation of inflammation. Up to very recently, the IL-1 family cytokines appeared to include inflammatory cytokines (such as IL-1 and IL-18) that activate target cells through a receptor-mediated mechanism shared with TLR receptors, and one anti-inflammatory cytokine antagonist (IL-1Ra) capable ofblocking IL-1-dependent activation by mechanical occupation of the receptor (Debets *et al.,* 2001) .

1.2.7.1.4 The Role of IL-37 in Asthma.

a member of the IL-1 cytokine family, in allergic asthma. It has been shown that respiratory bacterial and viral infection can be provoke allergic inflammation in allergic asthma (Schröder *et al.,* 2008) and (Ritchie *et al.,* 2015) . The regulatory cytokine IL-37b , the largest and best characterized

variant of IL-37, is an immunosuppressor that exerts anti-inflammatory activity in inflammatory diseases (Nold *et al.,* 2010) .

It has been found that the IL-37 has five different splice isoforms, named IL-37a, IL-37b, IL-37c, IL-37d, and IL-37e, according to the encoded exons (Lambrecht B.N. *et al.,* 2015) . Resent study showed the first time that IL-37a subtype can exert significant anti-inflammatory effect in chronic allergic asthma .IL-37a and IL-37b can improve airways structural changes including mucus expression, collagen deposition, hypertrophy of smooth muscles and neovascularization of chronic allergic asthma mice model. IL-37a and IL-37b subtypes might have therapeutic effects on asthma, possibly through attenuating airways inflammation and remodeling, which provides new insights for the treatment of asthma (CUI, Lele, *et al.,* 2023) .

In similar study suggest that both IL-37a and IL-37b isoforms are able to not only ameliorate airways inflammation and airways hyperresponsiveness, but also greatly reduce airways structural changes of animal models of chronic asthma. IL-37a and IL-37b subtypes might have therapeutic effects on asthma, possibly through attenuating airways inflammation and remodeling, which provides new insights for the treatment of asthma. the anti-inflammatory IL37 was down-regulated both in non-responders and in individuals with severe allergy. In accordance, strongly down-regulated IL37 levels associated with high levels of Th2 cytokines in individuals with severe allergy (Meisser *et al.,* 2024). IL-37 could be an important cytokine in the control of asthma by suppressing the production of inflammatory cytokines (Charrad *et al.,* 2016) .

1.2.7.2 Produce and Synthases of IL-38 .

IL-38 is predominantly expressed by keratinocytes and B–lymphocytes, with limited expression in other cellular sources. (de Graaf *et al.,* 2022). IL-38 is localaized within the IL-1 cluster on chromosome 2q13-14.1 (Bensen *et al.,* 2001). IL-38 is found mostly in the basal epithelia of the human skin and numerous other tissues, including nonimmune organs (heart, placenta) and immune ones (fetal liver, spleen, thymus, and activated B cells of the human tonsil) (de Graaf *et al.,* 2021) . Many IL-1 family cytokines are released into the extracellular space in response to cell stress or cell death (Kim *et al.,* 2016).

1.2.7.2 .1 IL-38 Discovery and Protein Structure .

About 20 years ago, the biomarker known as interleukin 38 (IL-38), formerly known as IL1HY2, was found. It is made up of 152 amino acids and belongs to the IL-1 family. The skin, tonsils, fetal liver, spleen, thymus, and salivary glands all routinely express IL-38 (Lin *et al.,* 2001 and Bensen *et al.,* 2001 and Ciccia *et al.,* 2015). The structural makeup of IL-38 consists of 12 β-strands joined by 11 loops, arranged in a β-trefoil pattern.

1.2.7.2 .2 IL-38 receptors and signaling

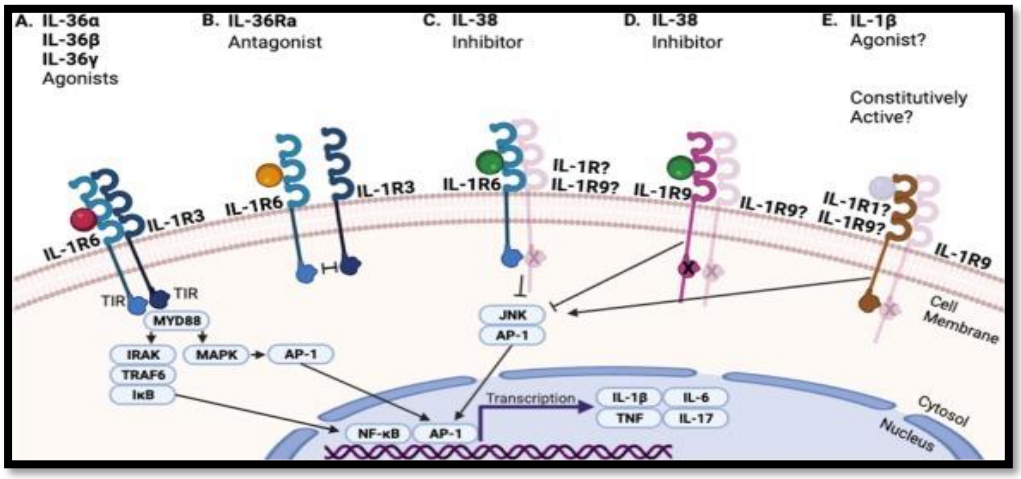


Fig. (1.4): IL-38 Receptors and Signaling (De graaf *et al.,* 2022).

1.2.7.2 .3. Characteristic And Impotant Of Increased Serum Interleukin 38 (IL-38) .

(Wu *et al.,* 2018) state that because most members of the IL-1 family regulate the expression of genes associated with inflammatory illnesses, they are considered pro-inflammatory cytokines. However, recent studies have demonstrated that IL-38 functions as an antagonist against the inflammatory cytokine IL-36 and prevents the production of inflammatory cytokines from macrophages, thereby having an anti-inflammatory effect (Wu and Garraud, 2018; Wu *et al.,* 2018) . Chu studies show a significant increase in serum levels of IL-38 in people with pediatric asthma. Furthermore, they found a strong negative correlation between serum levels of IL-38 and the fraction of Treg cells in people with elevated periostin levels—which is believed to be a unique blood biomarker of airway eosinophilia (Chu *et al.,* 2011).

The in vitro actions of IL-17, IL-22, IFN-γ, and IL-36γ suppress keratinocytes expressing high amounts of endogenous IL-38 (L. Mercurio *et al.,* 2018). but endogenous IL-38 is not substantially expressed in resting PBMC and can be activated by IL-17, TNF, and IFN-γ (H.J. Kim *et al.,* 2016). These innate inflammatory cytokines exhibit a surprising yet explicable differential regulation of IL-38. IL-38's persistent anti-inflammatory effects may naturally help the skin barrier maintain homeostasis (Lachner J. *et al.,* 2017).

1.2.7.2 .4. IL-38 in Asthma

IL-38 may prove to be a valuable novel biomarker for atopic asthma, especially in younger patients. For patients with eosinophilic asthma who may not be producing enough of the cytokine IL-38 naturally, it can offer a novel avenue of biological treatment to counteract and decrease the inflammatory responses and eosinophil accumulation in the airway system. To clarify the precise function and mode of action of IL-38 and to confirm that it can be used as a treatment alternative for individuals with severe eosinophilic asthma, more research, experimentation, and clinical trials are required (Kamal *et al.,* 2022) **.**

In a particular study, we discovered that the children with asthma had much greater serum levels of IL-38 than the control group of healthy individuals. Furthermore, only in the atopic group did the serum levels of IL-38 show an inverse correlation with the degree of eosinophilia, indicating that IL-38 serum levels may serve as a biomarker for children patients with atopic asthma (Kamal *et al.,* 2022) **.**IL-38 may be a potential cytokine in the management of allergic disorders, according to a recent review (Tsang *et al.*, 2020) on the significance of IL-1 family cytokines in the development and pathogenesis of allergic diseases. The purpose of such study was to further elucidate the possible involvement of IL-38 in pediatric patients' bronchial asthma.

According to(Bosnjak *et al.,* 2011), asthma is defined by a Th2 response that causes eosinophilic inflammation, excessive mucus formation, and airway constriction. Similar to periostin, IL-5, IL-6, IL-13, IL-17, IFN–γ, and IL-1β, and regardless of steroid treatment, IL-38 blood concentrations were considerably higher in children with asthma compared to control patients (Chu *et al.,* 2016).

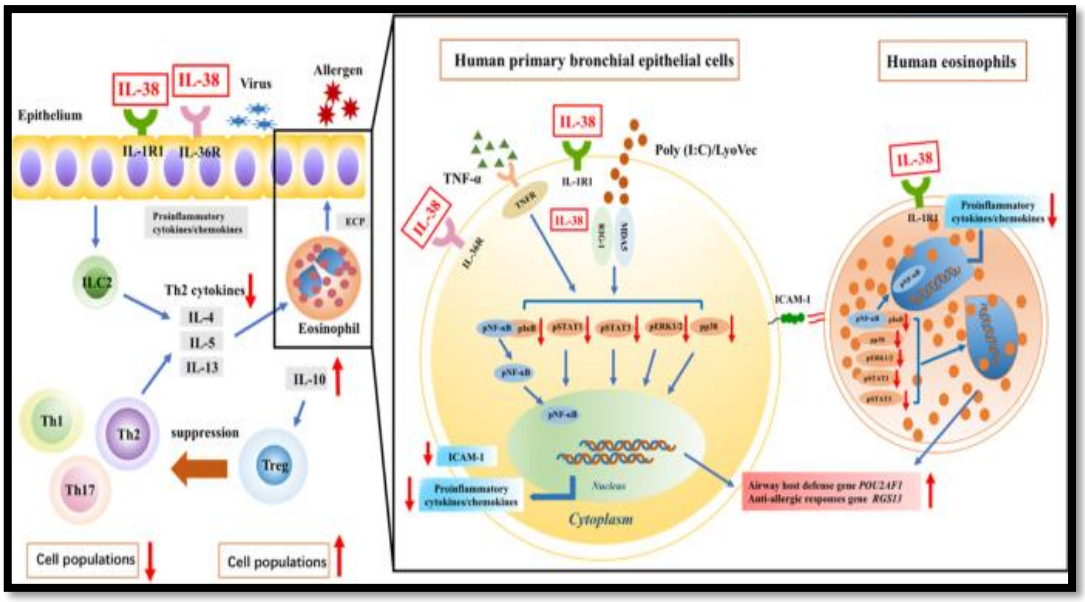


Figure (1.5) : Anti-inflammatory activities of IL-38 in allergic airway inflammation(SUN, Xiaoyu, *et al.*, 2020).

1.2.8. Both IL-37 and IL-38 .

Innate inflammatory cytokines stimulate the production of both IL-37 and IL-38, which then operate in a negative feedback loop to downregulate a group of related cytokines. Reduced expression of resting mRNA suggests that the host should first undergo innate defenses against infection before IL-38 and IL-37, two anti-inflammatory cytokines, are produced to reduce inflammation.(Graaf *et al.*, 2022). Now... As a member of the IL-1 family, IL-38 has anti-inflammatory characteristics similar to those of IL-37 (G. Cavalli *et al.*, 2018) **.**

Interestingly, the anti-inflammatory IL37 was significantly down-regulated in individuals with severe allergy and non-responders, whereas the anti-inflammatory IL38 (IL1F10) was not significantly affected in any of the groups although four out of five individuals with severe allergy strongly down-regulated IL38 . (meisser, Sanne S., *et al.*, 2024.)

1.2.9. Diagnosis Asthma:-

There is no gold standard single test to make a diagnosis of asthma, there are several objective tests that can be used to support the diagnosis including physiological measures such as obstructive spirometry associated with bronchodilator reversibility and airway hyperresponsiveness. In addition, non-invasive tests of airway inflammation such as exhaled nitric oxide or peripheral blood eosinophils are important to identify those with an allergic or eosinophilic phenotype (Saglani and Menzie-Gow, 2019)

1.2.9.1 Phenotypes of asthma:-

At the first we must distinguish between phenotype and endotype. Phenotypes are defined as "observable characteristics that result from a combination of hereditary and environmental influences." While asthma endotypes describe these distinct pathophysiologic mechanisms at a cellular and molecular level.

A number of different phenotypes can be described. Distinguishing between them can be particularly relevant to the therapy in severe cases:

1. **Allergic (extrinsic) asthma :** Atopic asthma most common type of asthma in the pediatric age and is distinguished by eosinophilic airway inflammation associated with specific immunoglobulin E (IgE) antibodies sensitization to various allergens, as evidenced by serology or skin prick test(Froidure *et al.*, 2016).Patient with atopic asthma will have mast cell-bound IgE molecules residing in their airways. Inhalation of the offending allergen leads to cross-linking of adjacent IgE molecules, causing mast cell activation and release of mediators including histamine and tryptase. This leads to an immediate or acute-phase asthmatic reaction, peaking at 15 minutes and resolving within an hour. Around 50% of asthmatics also

experience a late-phase reaction at about six hours, due to a Th2 lymphocyte-mediated influx of inflammatory cells, eosinophils in particular, and further release of mediators(Ali, 2011)

2. **Non allergic (intrinsic) asthma:** The definition of non-allergic asthma includes that subset of subjects with asthma and with whom allergic sensitization cannot be demonstrated. These individuals should have negative skin prick test or in vitro specific-IgE test to a panel of seasonal and perennial allergens. Non allergic asthma occurs in 10% to 33% of individuals with asthma and has a later onset than allergic asthma, with a female predominance (Peters, 2014).

1.2.9.2 History:

The most information is given by the parents. Engaging in play is important for a child's normal social and physical development, but physical activity is a substantial trigger for asthma symptoms, so children often abstain from strenuous play or exercise to avoid symptoms (Papi *et al.*, 2018)

1.2.9.3 Lung Function Testing:

Spirometry is a physiological test that measures how an individual inhales or exhales volumes of air as a function of time. The primary signal measured in spirometry may be volume or flow(National Institute for and Care, 2017). To confirm the diagnosis if an airway obstruction is found reversible based on an FEV_1 (Forced expiratory Volume in 1 second) increase of >12 % and >200 ml after administering 200–400 µg salbutamol(Horak *et al.*, 2016).

1.2.9.4. Laboratory Tests :

Among many laboratory tests readily available in most of referral centers, few are recommended to use for the diagnosis of asthma :

1.Total immunoglobulin E (Total IgE) levels has been shown to be major contributing factor for the development of bronchial hyperresponsiveness in asthma. An elevation in serum IgE levels contributes to asthma and is considered a potent predictor of the development of asthma(Lama *et al.,* 2013).

Total serum IgE (tIgE), allergen-specific IgE (sIgE) are commonly used markers for atopy and atopic disease(Vereecken *et al.,* 2012). Serum total IgE does not meet the criteria of an ideal biomarker. As an indicator of allergic asthma it is appropriate , it may be present in other allergic, parasitic and immune diseases. Similar to other biomarkers, tIgE may be an indicator of a biological (atopy) or pathological process (allergic disease)(Sánchez-García *et al.,* 2017).

2- eosinophil count test: High blood eosinophil counts are a modest predictor of future exacerbations, and may predict a favorable response to Inhaled corticosteroids (ICS), especially in patients with a history of frequent exacerbations and it is important to identify patients who may benefit from treatments in airways disease is important in order to reach informed treatment decisions for the maximal benefit of patients(Barnes *et al., 2016)*. Patients with asthma and blood eosinophil counts greater than 400 cells per μL experience more severe exacerbations and have poorer asthma control(Price *et al.,* 2015). So blood eosinophils can help predict the risk of exacerbation, could help guide more optimal treatment plans and reduce cost (Casciano *et al.,* 2016).

1.2.10. Treatment of asthma:

The degree of illness control and the intensity of the symptoms were factors in the treatment of asthma. Corticosteroids, leukotriene modifiers, and beta-2 adrenergic agonists are the three pharmacological types most

frequently utilized in asthma treatments. Treatment for severe asthma has included intramuscolar triamcinolone. Although xanthines and chromones have been utilized widely in the past, their efficacy and safety profile have exhibited limitations. Omalizumab is a monoclonal antibody against IgE that is utilized as a novel immunomodulatory biological therapy in patients with verified IgE-mediated allergic asthma, but only within the patient's particular range of total IgE level (Tesse *et al.,* 2018) **.**

1.2.10.1. oxygen:

A titrated oxygen regime is recommended in the treatment of severe asthma, in which oxygen is administered only to patients with hypoxaemia (Perrin *et al.,* 2011)

1.2.10.2. Inhaled corticosteroids:

ICS are considered the most effective drugs used to suppress airway inflammation in asthma. However, Inhaled medications are only effective if they are used properly, Although ICS treatment is generally considered safe in children(Hossny *et al.,* 2016). Corticosteroids are the most effective treatment for asthma; inhaled corticosteroids (ICSs) are the first-line treatment for children and adults with persistent symptoms(Ramadan *et al.,* 2019).

1.2.10.3.Allergy immunotherapy :

Allergy immunotherapy (AIT) is an effective treatment for allergic asthma and rhinitis. In addition to reducing symptoms, AIT can change the course of allergic disease and induce allergen-specific immune tolerance(Burks *et al., 2013).*

1.2.10.4 . Montelukast:

montelukast is effective on many biological and pathophysiological mechanisms involved in asthma, and on which ICS are only partially effective . It represents a good alternative to ICS as immunotherapy, and it is a particularly good additional treatment to ICS in large groups of patients, with the aim of reaching and maintaining control of asthma with the minimal possible dose of ICS(Paggiaro and Bacci, 2011). It has proven efficacy in reducing asthma exacerbations, but the effect size of montelukast (a leukotriene receptor antagonist) for varied severity of asthma exacerbations is not systematically assessed(Zhang *et al.*, 2014).

Chapter Two

Materials and Method

2.1. Study Design and Setting :

This is a case –control study that was done at teaching children hospital in Karbala,. All patients were registered in respiratory center in hospital from august (2023) to january (2024).

2.2. Subjects Group :

One hundered (100) participants were enrolled in this study including two groups involved in this case-control study according to clinical diagnosis by a clinician: the first group it is patien 50 individual . The second group 50 individual healthy control group. all of the groups ages range from 1 to 5 years **.** detailed case information sheets involving age, gender, full history and other variables were carried out for each patient by a questionnaire as in (appendix 1).

2.3 : Basic Design Of The Study

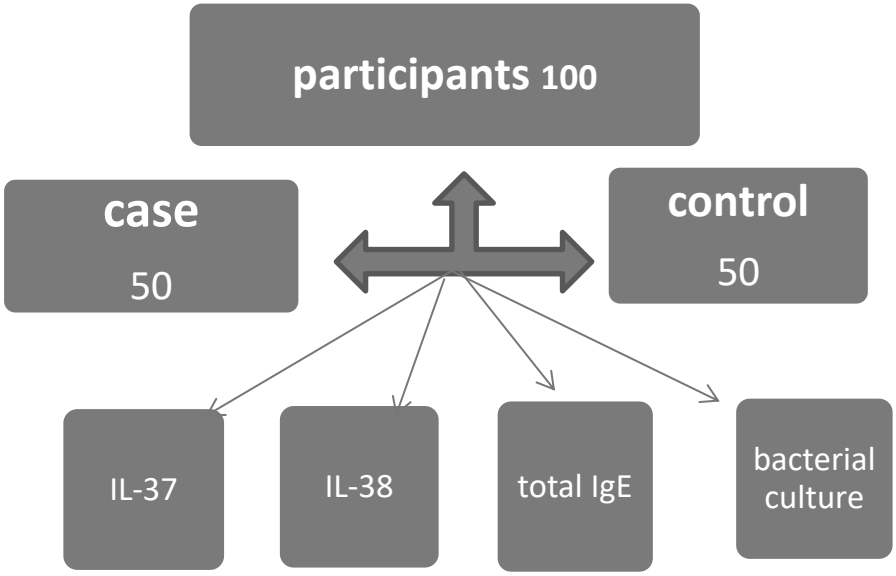
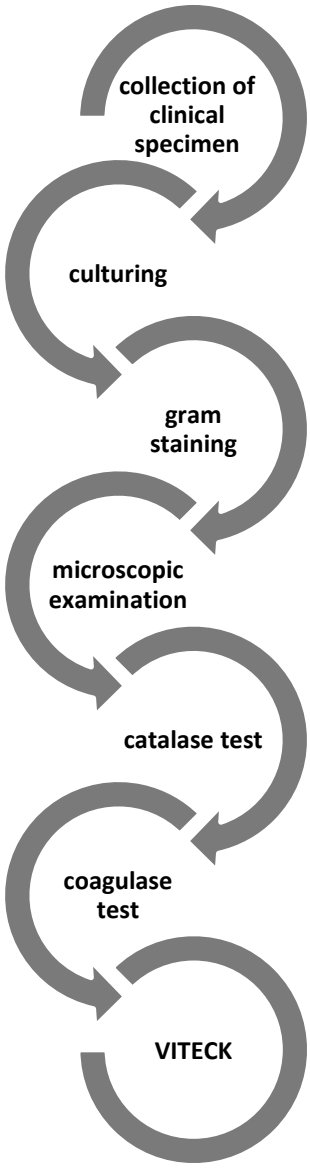


Figure 2.1 : : Basic Design Of The Study

2.3.1. Bacterial Study Design:



2.4. Inclusion Criteria: Patients' inclusion criteria were age 1-5 years old; both sexes, diagnosed as asthma\recurrent wheezer

2.5. Exclusion Criteria:patients were excluded if infection in the respiratory tract is suspected, antibiotic treatment up to 2 weeks ago, any other inflammatory or autoimmune diseases, and patients on drugs with immunosuppression.

2.6. Designation of Questionnaire for Patients:

Those patients were registered at teaching children hospital in Karbala,. All patients were registered in respiratory center in hospital and were questioned with a specially designed questionnaire. This questionnaire contains demographic data including age , gender , live ,telephone numbe , Family history of asthma allergic rhinitis, cough,sneezing,shorten in breth , passive smoking , Usage of other drugs, tepe of asthma severity, found animal in house , type of treatment, allergic food or drug . Results CBC (lymphocyte and neutrophis) and eiosinophil count were collected after blood drawing (appendix 1**).**

2.7. Ethical Approval:

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

2.8. Materials:

2.8.1. Equipment and Instruments Utilized in the Study:

In the present study, the following equipment and instruments were used in table (2.2).

Table (2.1): Devices and Instruments. Equipment & Instruments Manufacturing Company Origin.

Equipment & Instruments Manufacturing	Manufacturing Company	Origin
Autoclave	Hirayama HVE-50	Japan
Incubator	Memmert	Germany
Refrigerator	Panasonic	Korea
Para-film	Bemis	USA
water distillatrory	GFL	Germany
VITEK 2 compact system	Bio merieux	France
Syringe 3 ml	Arrow	Egypt
Centrifuge	Kokusan	Japan
a micro-pipette.	SLAMED	Germany
pipette tips.	ALS	China
a drying rack.	. ALS	. China
Deep freezer	Hettich	Korea
Light microscope	Olympus	Japan
a sterile glass (gel tube)&(plain tube)	ALS	China
well-fitting gloves.	ALS	China
Burner	Amal	Turkey
a tourniquet.	Himedia	India
Slides	Himedia	India
70% alcohol swabs for skin disinfection.	trest	chins
gauze or cotton-wool ball to be applied over puncture site.	BDH	England
maker	trest	china
Swab media	Himedia	India
flasks (different size)	Jlassco	India

Biological safety cabinet	**EuroCloneSafemate**	**Italy**
Loop	**Himedia**	**India**
Water bath	**Polyscience**	**USA**
Multichannel micropipette set	**SLAMED**	**Germany**
ELIZA Devices (washer & reader)	**Human**	**Germany**
ELIZA printer	**Epson**	**Japan**
vortex	**Clay Adams**	**Germany**
Sensitive balance	**Sartorius**	**Germany**

2.8.2. Chemicals and Biological materials :

The chemicals and biological materials are listed in table (2.3).

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

Chemicals and biological materials	Company	Country of origin
Normal saline (0.9	**choueifat**	**Lebanon**
Gram's stain kit	**Biolife**	**Italy**
Glycerol	**Biolife**	**Italy**
Oil immersion	**BDH**	**England**

2.8.3. 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**
Brain heart infusion broth (BHIB)	**Oxoid**	**England**
MacConky agar	**Oxoid**	**England**
Manitol salt agar	**Oxoid**	**England**

2.8.4.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
Human Interleukin 38 (Interleukin 37) ELISA Kit	**BT LAB / China**
Human IL-37 (Interleukin 37) ELISA Kit	**BT LAB / China**
Total IgE ELISA Kit	**Demeditic**
ID (VITEK2) cards cassette	**BioMerieux**

Table (2.5): Reagents and Quantity (IL-38) ELISA Kits.

Components	Quantity
Standard Solution (240ng/L)	**0.5ml x1**
Pre-coated ELISA Plate	**12 * 8 well strips x1**
Standard Diluent	**3ml x1**

Streptavidin-HRP	**6ml x1**
Stop Solution	**6ml x1**
Substrate Solution A	**6ml x1**
Substrate Solution B	**6ml x1**
Wash Buffer Concentrate	**(25x) 20ml x1**
Biotinylated Human IL-38 Antibody	**1ml x1**
User Instruction	**1**
Plate Sealer	**2 pics**
Zipper bag	**1 pic**

Table (2.6): Reagents of Human IL-37 ELISA Kits

Components	Quantity
Standard Solution (240ng/L)	**0.5ml x1**
Pre-coated ELISA Plate	**12 * 8 well strips x1**
Standard Diluent	**3ml x1**
Streptavidin-HRP	**6ml x1**
Stop Solution	**6ml x1**
Substrate Solution A	**6ml x1**
Substrate Solution B	**6ml x1**
Wash Buffer Concentrate	**(25x) 20ml x1**
Biotinylated Human IL-37 Antibody	**1ml x1**
User Instruction	**1**
Plate Sealer	**2 pics**
Zipper bag	**1 pic**

Table (2.7): 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**

2.9. method:

2.9.1 Biological Sampling:

2.9.1.1. Specimen Collection of nasopharngeal swab .

The NPS procedure was done by The patient head was tilted back slightly and swab was inserted into the naris until it reached the posterior nasopharynx. The gel swab has been shown to be retain microorganisim for three days (72 hour) The swab was rotated gently and then immediately placed into cover with gel . This procedure was repeated with the other naris. Multiple media prepared for culturing. NPS specimens were cultured on blood agar and chocolate agar and macconkey agar and mmanitol salt agar , after culturing can incubated for 18- 24 hour in 37^C in laboratory .

2.9.1.2. Blood Sample.

Venepuncture is the preferred method of blood sampling , Use a syringe with a barrel volume of 3 ml, depending on collection needs; the vacuum produced by drawing using a larger syringe will often collapse the vein. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible.

2.9.1.2.1 Select the Site.

• Stretch out the patient's arm and look at the forearm or antecubital fossa.
• Identifies a good-sized vein that is apparent, straight, and clear.
• Depicts the typical locations of the vessels, however there are many conceivable deviations. The most common vein to puncture is the median cubital vein, which is located in the space between muscles. A haematoma is more likely to occur when a needle is inserted in a location where veins are diverting.Without using the tourniquet, the vein ought to be visible. The right needle size can be chosen with the help of finding the vein.
• Recheck the vein and apply the tourniquet 4-5 finger widths above the venipuncture site.
• Wipe the area with a 70% alcohol swab for 30 seconds before drawing blood or getting ready for a blood collection, then give it time to dry completely.

2.9.1.2.2. Perform venepuncture as follows.

• Holding the patient's arm, place a thumb below the venepuncture site to anchor the vein.

• To make the veins more noticeable, ask the patient to make a fist.
• Insert the needle into the vein as quickly as possible, ideally at a 30

degree angle or less, and keep going down the vein at the simplest angle to enter.

• Before removing the needle, relax the tourniquet once enough blood has been drawn.

• Gently remove the needle and use a clean gauze or dry cotton-wool ball to gently press on the area. Request that the patient elevate and extend their arm to hold the gauze or cotton wool in place. Request that the patient not bend their arm because doing so can result in a hemorrhage.

2.9.2. Laboratory Methods

2.9.2.1. Serum preparation

After Draw a sufficient amount of whole blood into a serum gel tube. Gently invert the tube several times after collection to activate clotting. Allow blood to clot at ambient temperature for 20 to 30 minutes. Centrifuge for 5 minutes to separate the serum from clot and transfer the serum to a screw-capped, plain tube This process should be completed within 1 hour of collecting the specimen for optimal results.

2.9.2.2. Culturing the Sampling.

2.9.2.2.1. culturing Media (Streak method) :

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**.**

Pure Culture: Culture consist of medium containing the growth of a single species of M.O which involve in most microbiological studies and industries.

Mixed Culture: Culture consist of medium containing the growth of two or more species of M.O. There are several ways to ensure the purity of a culture.

Is the mixture of various nutrients that is suitable for the growth of microorganisms. this media should provide bacteria with most of important nutrition requirements for growing such as water, source of carbon , energy , nitrogen , carbohydrates , amino acid , inorganic salts , particular growth factors in some time like vitamin (K) and others . these nutrients are supplied by aqueous extracts of meat and peptone .

2.9.2.2.2. types of media used in method of culturing

A: Blood Agar

A nonselective medium for the growth of harmful bacteria, such as *streptococci*, is blood agar. It also acts as a medium for differentiation between hemolytic and non-hemolytic microorganisms. Hemolysis is often permitted in the medium. Cytolytic toxins are typically produced by hemolytic bacteria. In blood agar, these toxins have the power to lyse red blood cells. As a result, the medium receives the red component of the cells. The medium becomes stained or dark due to hemolysis. Actually, there are three different kinds of hemolysis: gamma hemolysis, which leaves the medium uncolored, beta hemolysis, which turns the medium clear, and alpha hemolysis, which turns the medium dark or stained.**as in appendix 4 ,**Furthermore, blood agar is a red color medium that is opaque. Blood agar is an enriched medium that contains an array of uncommonly rich nutrients.

B: chocolate agar

Another nonselective, enriched medium that's crucial for isolating harmful bacteria is chocolate agar. The primary characteristic of chocolate agar is

the presence of heated red blood cells that have been lysed. Chocolate agar is essential for the growth of bacteria that are picky eaters, like *Neisseria meningitidis* and *Haemophilus influenzae*. These bacteria typically need two internal growth factors found in red blood cells to proliferate. Factor V and Factor X are among them. Nicotinamide adenine dinucleotide is factor V; hemin is factor X. Therefore, the primary ingredient needed for these bacteria to develop is red blood cell lysate.

Definition: Blood agar refers to a general-purpose, enriched media to grow fastidious organisms and differentiate bacteria based on their hemolytic properties, while chocolate agar refers to a nonselective, enriched growth medium used for the isolation of pathogenic bacteria.

Red Blood Cells : Blood agar contains red blood cells of whole sheep blood, while chocolate agar contains red blood cells lysed by heating.

Color :Blood agar is red in color, while chocolate agar is brown to brownish red in color.

Cooling Temperature :Sterile sheep blood is added at 50°C to nutrient agar in the preparation of blood agar, while sterile sheep blood is added at 75-80°C to nutrient agar in the preparation of chocolate agar.

Factor V and X :Factor V and X occur inside the red blood cells in blood agar, while Factor V and X occur in the medium in chocolate agar.

Type of Organisms :Blood agar is important for the growth of *S. pyogens* and other *streptococcus* species., while chocolate agar is important of *Neisseria* and *Haemophilus* species

C: MacConkey Agar

Differential and selective media; *Enterobacteriaceae* identification contains peptone, lactose, and bile salts, which inhibit most Gram+ bacteria with the exception of *Enterococcus* and certain *Staphylococcus* species.

Indicators and Reagents: contains neutral red dye, which gives microorganisms that are fermenting lactose (and lowering pH) a pink stain, as well as crystal violet and bile salts, which suppress Gram+ bacteria.

Mechanism/reactions: *Enterobacter, Klebsiella,* and *Escherichia coli* are examples of Lac+ bacteria that use the lactose in the medium to create acid. This lowers the pH of the agar below 6.8 and causes red or pink colonies to develop. Since they are unable to use lactose, bacteria that do not ferment lactose, such as *Shigella*, *Proteus* species, and *Salmonella*, will instead use peptone. This results in the creation of ammonia, which elevates the agar's pH and causes white or colorless colonies to grow.

Interpretation:

(+) = Lactose fermentation, re/pink colonies

(-) = non-lactose fermenters, white/colorless growth

D: Mannitol Salt Agar

Differential and selective; pathogen detection The *staphylococci* include Mannitol, 7.5% Sodium Chloride, and Phenol Red in Mannitol Salt Agar (MSA)

Indicators and Reagents: Red phenol

Mechanism/reactions : Because the concentration of salt inhibits the majority of other organisms, the medium is *staphylococci*-specific. When *Staphylococcus aureus* ferments mannitol, the medium's pH changes to an acidic state, which may be seen by the color of phenol red turning yellow..**appendix 4**

Interpretation:

(+) = Growth and yellow halo surrounding it (also record growth/no color)

(-) = No growth, no color change

2.9.3. maintenance microbiota using brain heart infusion (BHI) broth.

2.9.3.1. Princiole of BHI .

BHI Medium is a highly nutritive medium that is used to cultivate a wide range of microorganisms. Both fastidious and non-fastidious microorganisms can be grown in BHI Broth, an enhanced non-selective broth medium. Aerobic bacteria from a range of clinical and non-clinical specimens will also grow well in this medium. BHI is utilized to maintain microbiota for three months in deep freezing, allowing for the culture of bacterial samples on nutrient or blood agar for eventual Viteks system diagnosis. **Appendix 5**

2.9.3.2. component of BHI.

The mixture of brain and heart infusions provides organic nitrogen, carbon, and vitamins. Dextrose is the carbohydrate source. A low concentration of dextrose is used to stimulate early growth. Sodium chloride maintains the osmotic environment. Disodium phosphate is the buffering agent in this medium and also helps neutralize the acids produced from the utilization of dextrose, thus maintain viability (Catrambone *et al.,* 2021).

2.9.4. Gram Stain

2.9.4.1. Preparation of Smear.

Fixation: The smear needs to be fixed before staining in order to coagulate the cell's protoplasm and ensure that the smear sticks to the slide, which will help the sample absorb the stain more easily. The slide can be fixed by holding it three to five times for three to four seconds over a Bunsen flame or by letting it dry in the air for fifteen to thirty minutes.

2.9.4.2. Gram Stain Component.

- Primary stain: they are basic dyes with chromatophores that are positively charged. Because chromatophores and bacteria are attracted to each other,

basic dyes stain all bacterial cells. The fundamental dyes crystal violet, methyl violet, and gentian violet are a few that can be utilized as main stains.

• Mordant: Iodine serves as a mordant and is added to improve the dye's attraction for bacteria. It attaches the dye to the slide by forming a combination with crystal violet, an insoluble material in the cytoplasm.

• Decolorizer: it takes away the main stain from cells that have been stained. The most used decolorizer is acetone, which is followed by ethanol.

• Counterstain: a simple dye that contrasts with the primary stain in color. Counterstains such as neutral red, diluted carbol fuchsin, and safranin can be employed.

2.9.4.3. Interpretation of Gram Staining Results.

Under a microscope, unstained bacteria appear opaque. Staining is necessary to increase the contrast between the organism and the backdrop, which makes the cells and their internal features easier to see under a light microscope. Two types of staining procedures exist.A basic staining technique uses a single stain and covers the entire cell in the same color. There is a small negative charge in bacterial cells. Direct staining, also known as positive staining, is the process of directly staining bacterial cells using simple stains with a positive charge, like crystal violet or methylene blue. When using acidic dyes like nigrosin or Indian ink, just the background is stained and the organisms look translucent. This technique is known as indirect or negative staining.While Gram-positive bacteria stain purple, Gram-negative bacteria will appear red (Vijayakumar *et al.*, 2023) .

2.9.4.4. Factors Affecting Staining.

• The age of the bacterial culture: Older cell cultures (those that have been around for more than 24 hours) quickly become Gram-negative and lose their gram-positivity.

• Heat: During fixation, too much heat may cause Gram-positive cells to disappear. The cell wall is weakened by excessive heat. Gram-positive bacteria start to take on characteristics of Gram-negative bacteria.

• Overcrowding of cells: An excessive number of cells might cause incorrect decolorization, which affects the gram staining procedure. When the cell becomes congested, thin streaks become discolored more quickly than thick stains. Gram-positive bacteria turn become Gram-negative bacteria once the cell wall is broken(Vijayakumar *et al.*, 2023)

2.10. Sterilization Methods

A: Sterilization of the culture media used in the present study by autoclave at 121Co for 15 minutes.

B. Sterilization of the glass wares are done by dry heat in an electric oven at 180 Co for 2 h.

2.11. Bacterial Profile identification

2.11.1. Morphological Tests

Colonial characteristics were tested such as the shape of the colonies, size, color, borders, and texture of colonies.

2.11.2.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 (Tille, 2017).

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

The fastest and most precise techniques for identifying bacteria are automated ones. In order to provide a biochemical profile that is utilized for organism diagnosis, the VITEK2 system is made up of plastic reagent cards with microliter quantities of various biochemical test media in 30 wells (Maina & Kagotho, 2014). A photometer is used to evaluate the color changes in the card caused by the microbe's metabolic activity. An inoculum obtained from cultured samples is automatically placed into the card. A computerized data base was used for the analysis, storing, and printing of the data. Various card kinds, such as those for Gram-positive and Gram-negative identification (GN and GP), are available (Maina & Kagotho, 2014). The phases of the VITEK 2 compact system indicated in (**appendix 6**).

2.12. Maintenance of bacterial isolates:

The bacterial isolates' maintenance was done as the following

2.12.1. Short-Term Storage

The pure bacterial isolates were maintained for a few months in screw-capped universal tubes containing brain heart infusion agar slant, and

incubated at 37ºC for 24 hours. The slants were tightly wrapped with Parafilm and then kept at 4ºC for three months (Benson, 2002.)

2.12.2. Long-Term Storage

A brain heart infusion broth was inoculated by a loop of overnight pure bacterial culture and incubated at 37°C after 18 hours. Glycerol was added to inoculate in the final concentration (20%) and stored at -20°C for 2-8 months (Green, 2015).

2.13. immunological markers

1- IL-37

2- IL-38

3- total IgE

2.13.1. Determination of the level of Human Interleukin 38 (Cat. No E3276Hu) appendix 7:

2.13.1.1. Principle of Sandwich ELISA Technique:

This kit is an enzyme-linked immunosorbent assay (Elisa). The plate has been pre-coated with a human il-38 antibody. Il-38 present in the sample is added and binds to antibodies coated on the wells. There, biotinylated human il-38 antibody is added and binds to il-38 in the sample. Then streptavidinhrp is added and binds to the biotinylated il-38 antibody. After incubation unbound streptavidin-HRP has washed away during a washing step. A substrate solution is then added and color developed in proportion to the amount of human il-38. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

2.13.1.2. Reagent preparation

1. All reagents brought to room temperature before use.

2. Standard reconstituted the 120µl of the standard (240ng/l) with 120µl of standard diluent to generate a 120ng/l standard stock solution. Allowed the standard to site for 15 minutes with gentle agitation prior to making dilutions. Prepared duplicated standard points by serially diluting the standard stock solution (120ng/l) 1:2 with standard diluent to produce 60ng/l, 30ng/l, 15ng/l and 7.5ng/l solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution had frozen at -20°c and used within one month. Dilution of standard solutions suggested are as the following

Wash buffer: diluted 20ml of wash buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x wash buffer. Where noticed if the crystals had formed in the concentrate, mixed gently until the crystals had completely dissolved.

2.13.1.3. Assay procedure:

1. Prepared all reagents, standard solutions, and samples as instructed. Brought all reagents to room temperature before use. The assay was performed at room temperature.

2. Determined the number of strips required for the assay. Inserted the strips in the frames for using. The unused strips were stored at 2-8°c.

3. Resereacher added 50µl standard to the standard well. Note: don't addition antibodies to the standard well because the standard solution contains a biotinylated antibody.

4. Addition of 40µl sample to sample wells and then we added 10µl anti-il38 antibody to sample wells, then added 50µl streptavidin-hrp to sample wells and standard wells (not blank control well). Mixed well and covered the plate with a sealer. Incubated 60 minutes at 37°c.

5. Resereacher removed the sealer and washed the plate 5 times with washing buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each washing. For automated washing, aspirate or decant each well and washed 5 times with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. 50µl substrate solution was added to each well and then adds 50µl substrate solution b to each well. Incubated plate covered with a new sealer for 10 minutes at 37°c in the dark condition.

7. Resereacher added 50µl stop solution to each well, the blue color would change into yellow immediately.**appendix 3**

8. Determined the optical density (od value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after addition of the stop solution.

2.13.1.4. Calculation of result

Construction of the standard curve by plotting the average od for each standard on the vertical (y) axis against the concentration on the horizontal (x) axis and draw a best fit curve through the points on the graph. These calculations could be best performed with computer-based curve-fitting software and the best fit line could be determined by regression analysis. The standard curve of il-38 in (appendix 2).

2.13.2. Determination the level of human il-37(interleukin 37) (cat. No: e-el-h2571) appendix 7:

2.13.2.1. Principle of sandwich Elisa Technique:

This kit is an enzyme-linked immunosorbent assay (Elisa). The plate has been pre-coated with a human il-37 antibody. Il-37 present in the sample is added and binds to antibodies coated on the wells. There, biotinylated human il-37 antibody is added and binds to il-37 in the sample. Then streptavidinhrp is added and binds to the biotinylated il-37 antibody. After incubation unbound streptavidin-hrp has washed away during a washing step. A substrate solution is then added and color developed in proportion to the amount of human il-37. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

2.13.2.2. Reagent preparation

1. All reagents brought to room temperature before use.

2. Standard reconstituted the 120µl of the standard (240ng/l) with 120µl of standard diluent to generate a 120ng/l standard stock solution. Allowed the standard to site for 15 minutes with gentle agitation prior to making dilutions. Prepared duplicated standard points by serially diluting the standard stock solution (120ng/l) 1:2 with standard diluent to produce 60ng/l, 30ng/l, 15ng/l and 7.5ng/l solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution had frozen at -20°c and used within one month. Dilution of standard solutions suggested are as the following:

Wash buffer: diluted 20ml of wash buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x wash buffer. Where noticed if the

crystals had formed in the concentrate, mixed gently until the crystals had completely dissolved.

2.13.2.3. Assay procedure:

1. Prepared all reagents, standard solutions, and samples as instructed. Brought all reagents to room temperature before use. The assay was performed at room temperature.

2. Determined the number of strips required for the assay. Inserted the strips in the frames for using. The unused strips were stored at 2-8°c

3. Resereacher added 50µl standard to the standard well. Note: don't addition antibodies to the standard well because the standard solution contains a biotinylated antibody.

4. Addition of 40µl sample to sample wells and then we added 10µl anti-il37 antibody to sample wells, then added 50µl streptavidin-hrp to sample wells and standard wells (not blank control well). Mixed well and covered the plate with a sealer. Incubated 60 minutes at 37°c.

5. Resereacher removed the sealer and washed the plate 5 times with washing buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each washing. For automated washing, aspirate or decant each well and washed 5 times with wash buffer. Blotted the plate onto paper towels or other absorbent material

6. 50µl substrate solution was added to each well and then adds 50µl substrate solution b to each well. Incubated plate covered with a new sealer for 10 minutes at 37°c in the dark condition.

7. Resereacher added 50µl stop solution to each well, the blue color would change into yellow immediately. **Appendix 3**

8. Determined the optical density (od value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after addition of the stop solution.

2.13.2.4. Calculation of result

Construction of the standard curve by plotting the average od for each standard on the vertical (y) axis against the concentration on the horizontal (x)

Axis and draw a best fit curve through the points on the graph. These calculations could be best performed with computer-based curve-fitting software and the best fit line could be determined by regression analysis. The standard curve of il-38 in (appendix 2).

2.13.3. Determination of the level of Total Immunoglobulin e (total IgE) (cat. No. Deige02):

2.13.3.1. Principle of Sandwich Elisa Technique:

The total ige elisa is based on the principle of the enzyme immunoassay (eia). A monoclonal mouse-anti-human ige antibody is bound on the surface of the microtiter strips. Undiluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate together with antihuman-ige-peroxidase conjugate. A sandwich complex between the serum ige and the two antibodies develops. After a 30 minutes´ incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then the substrate (tmb) solution is pipetted and incubated for 15 minutes, inducing the development of a blue dye in the wells. The colour development is terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The

concentration of the ige antibodies is directly proportional to the intensity of the colour.

2.13.3.2. Preparation of Reagents

Washing solution: dilute 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 monoclonal anti-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.

2.13.3. 3. Assay procedure

1. Prepare a sufficient amount of microtiter wells for the standards and samples in duplicate as well as for a substrate Blank.
2. Pipet 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. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.

4. Empty 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. Pipet 100 µl each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted

6. Cover 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, pipet 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.

2.13.3.4. 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), for a quantitative evaluation the absorptions of the standards are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. Alternatively the use of electronic device is possible. The results can also be calculated with normal programs for automatic data processing, i.e. 4 parameter, spline, logit-log. Any sample

reading greater than the highest standard should be diluted appropriately with zero standard and reassayed. The result has to be multiplied by the dilution factor . In the laboratory the standard curve should be established in each assay run.

2.14. Statistical analysis

The real statistics resource pack software for mac (release 7.2) of the resource pack for excel 2016 and the statistical package for the social sciences software, version 28.0 (ibm, spss, chicago, illinois, usa), were used to verify, manage, and analyze all of the study group's data. (2013–2020) copyright. The mean, standard deviation, frequencies, and percentages were displayed in descriptive statistics based on the categories of variables. Using the $\chi 2$-test and the t test to compare between groups, the data distribution was examined. Furthermore, to compare more than two groups, we employed the anova test.

Moreover, post hoc analyses using multiple pairwise comparisons and least significant difference (lsd) were used to compare each of the two groups. In order to evaluate the efficacy of il-37 and il-38 in separating disease from the control group and establishing the cutoff point, receiver operating characteristic curve analysis (roc) was employed.

Analytical statistical studies indicated significant variations in categorical variables among the parameters. All hypothesis test results with p-values less than 0.05 were deemed statistically significant.

ChapterThree

ResultS

3.1. Demographic Characteristic Of Study Groups :

There were 100 individuals enrolled in this study represented the two studied groups, 50 patient, 50 control. Baseline characteristics of the studied groups are shown in (Table 3.1), In the current study there were no-significant differences (P-value 0.683) , (p-value 0.343)between studied groups according to the age and sex when compared among them and with the control group. However, according to the Resident showed patient life in rural more connected with asthma than urban ($p < 0.005$). .

highly significant showed with patient have Eczema (p-value 0.002) where more individual (62.0)% have Eczema. Also individual with Allergic rhinitis/ conjunctivitis have highly significant (p-value 0.001) where (90.0)% of individual have Allergic rhinitis/ conjunctivitis. the results of this study showed that there highly significant differences (p-value 0.006) in relation to allergy Drug\ food for the patient's groups with Penicillin, Spiecy. Family history of asthma was significantly more frequent in patients with pediatric asthma (P. value = 0.001) where (64.0)% of child they gained asthma of family . Animal in house have dominant role in relationship with asthma , so approximately (64.0)% of individual that have cats, dogs, birdis, became more exacerpation with asthma ,significant differences (p-value 0.001). as for Passive smoking have highly significant (p-value 0.006) , where (72.0)% of individual they gained Passive smoking by another people .

Table 3.1. Baseline Characteristics Of The Studied Groups:

Table 1: Distribution and Characteristics of Patients and Control According to the study subjects.					
Variable	**Level**	**Control**	**Patient**	**Total**	**P-Value**
		Number(Percentage) %	**Number(Percentage) %**	**Number(Percentage) %**	
Age Group ≤ 5	**Less & equal than 3**	**21 (42.0) %**	**19 (38.0)%**	**40(40.0)%**	**0.683**
	Greater than 3	**29 (58.0) %**	**31 (62.0) %**	**60(60.0)%**	
Sex	**Female**	**29 (58.0) %**	**23 (46.0)%**	**52(52.0)%**	**0.343**
	Male	**21 (42.0) %**	**27 (54.0)%**	**48(48.0)%**	
Resident	**Urban**	**32 (64.0)%**	**18 (36.0)%**	**50(50.0)%**	**0.005*** **
	Rural	**18 (36.0) %**	**32 (64.0)%**	**50(50.0)%**	
Severity	**None**	**50 (100.0)%**	**0 (0.0)%**	**50(50.0)%**	**0.002*** **
	Mild	**0 (0.0)%**	**15 (30.0)%**	**15(15.0)%**	
	Moderate	**0 (0.0)%**	**23 (46.0)%**	**23(23.0)%**	
	Severe	**0 (0.0)%**	**12 (24.0)%**	**12(12.0)%**	
Treatment	**None**	**50 (100.0)%**	**0 (0.0)%**	**50(50.0)%**	**0.002*** **
	Montelukast	**0 (0.0)%**	**14 (28.0)%**	**14(14.0)%**	
	ICS	**0 (0.0)%**	**7 (14.0)%**	**7(7.0)%**	
	ICS, Montelukast	**0 (0.0)%**	**29 (58.0)%**	**29(29.0)%**	
Response	**None**	**50 (100.0)%**	**0 (0.0)%**	**50(50.0)%**	**0.001*** **
	Well	**0 (0.0)%**	**19 (38.0)%**	**19(19.0)%**	
	Not Well	**0 (0.0)%**	**22 (44.0)%**	**22(22.0)%**	
	Poorly	**0 (0.0)%**	**8 (16.0)%**	**8(8.0)%**	

	Severe	**0 (0.0)%**	**1 (2.0)%**	**1(1.0)%**	
Eczema	**Yes**	**0 (0.0)%**	**(3162.0)%**	**31(31.0)%**	**0.002*** **
	No	**50 (100.0)%**	**(1938.0)%**	**69(69.0)%**	
Allergic rhinitis/ conjunctivitis	**Yes**	**0 (0.0)%**	**45 (90.0)%**	**45(45.0)%**	**0.001*** **
	No	**50 (100.0)%**	**5 (10.0)%**	**55(55.0)%**	
allergy Drug\ food	**None**	**50 (100.0)%**	**25 (50.0)%**	**75(75.0)%**	**0.006*** **
	Penicillin	**0 (0.0)%**	**14 (28.0)%**	**14(14.0)%**	
	Spiecy	**0 (0.0)%**	**11 (22.0)%**	**11(11.0)%**	
History of asthma	**Yes**	**0 (0.0)%**	**(3264.0)%**	**32(32.0)%**	**0.001*** **
	No	**50 (100.0)%**	**18 (36.0)%**	**68(68.0)%**	
Animal in house	**Yes**	**0 (0.0)%**	**32 (64.0)%**	**32(32.0)%**	**0.001*** **
	No	**50 (100.0)%**	**(1836.0)%**	**68(68.0)%**	
Passive smoking	**Yes**	**0 (0.0)%**	**36 (72.0)%**	**36(36.0)%**	**0.006*** **
	No	**50 (100.0)%**	**(1428.0)%**	**64(64.0)%**	

The chi-square test has been utilized to analyze the categorical variables and Bonferroni Post Hoc Test explore which specific categories are significantly different

***. Association is significant at the 0.05 level.**

****. Association is significant at the 0.01 level.**

*****Bonferroni-adjusted significance level set at 0.0083 level.**

3.2 laboratory parameters of the studied groups :

3.2.1. lab paremeters distribution between patient and control.

The lab parameter level reported in both patient and control, mean was higher in patients other than control in all parameters as showed in table 2,significantly differences was appeared in IL-37 with studied groups (0.029) also important significant appear in IL-38 with studied groups (0.026) . so all blood parameter (lymphocyte , eosinophil , neutrophil) have highly significant with studied groups (p-value <0.001 , <0.001 , 0.023 respectively) . Serum total IgE levels were raised above the normal limits for the mean (626.5136) patients.

Table 3.2 : lab data distribution between patient and control

marker	name	N	Mean	Std. Deviation	p-value
IL-38 ng\L	Patient	50	89.3312	19.32555	0.026
	Control	50	45.0383	20.4521	
IL-37 ng\L	Patient	50	197.5	18.8	0.029
	Control	50	113.5	17.3	
total IgE IU\mL	Patient	50	626.5136	409.89627	NA
	Control	0[a]	.	.	
lymphocyte	Patient	50	8.5842	1.70114	<0.001
	Control	50	1.7778	.438361	
eosinophil	Patient	50	.9090	.21071	<0.001
	Control	50	.0.1804	.109058	
neutrophil	Patient	50	7.72126	1.907146	0.023
	Control	50	3.3582	1.235394	
Student T test p-value ≤ 0.05			NA: not applicable		

3.2.2. Correlation Laboratory Parameter Among Asthma Severity Class In Patient Group:

IL-37 and IL-38 serum level among asthma severity class in patient group where showed significant differences in IL-37 & IL-38 levels between asthma severity class in patient group. Although mean of IL-37 level increases in mild ,moderate ,severe asthma , (mean 172.31, 159.05, 134.08 respectively) unlike mean of IL-38 levels were less than IL-37 in asthma class (80.63, 85.01, 73.11).

Table 3.3 IL-37 and IL-38 serum level among asthma severity class in patient group

Severity		IL-37 ng\L	IL-38 ng\L
Mild	Mean	172.31	80.63
	N	15	15
	Std. Deviation	56.95	41.43
Moderate	Mean	159.05	85.01
	N	23	23
	Std. Deviation	73.66	34.47
Severe	Mean	134.08	73.11
	N	12	12
	Std. Deviation	65.21	29.93
Total	Mean	156.99	80.84
	N	50	50
	Std. Deviation	67.09	35.33
LSD		24.09	20.09
P value		0.034	0.054

3.3. Diagnostic utility of study parameters:

To assess the validity of IL-37and IL-38 as markers to predict complications of asthma, receiver operating characteristics (ROC) curve analysis was applied which revealed that both parameters, IL-37and IL-38were good predictors and markers of complications in PA. With regard to **IL-37**, in patient group , ROC curve analysis revealed that the area under the curve (AUC) was 80.40% which is a large area indicating higher prediction value at an optimal cutoff point of **IL-37** = 113.751 giving a high sensitivity, specificity and accuracy of 70.00% , 92.00% and 80.00%, respectively, with a Positive predictive value (PPV) of 89.47% and negative predictive value (NPV) of 74.19% , (Table 3.4 and Figure 3.1). For the **IL-38**, the validity parameters were not much different than that of **IL-37**in prediction of asthma At an AUC of 83.60% and a cutoff point of **IL-38**= 55.6100 , it was 78.00% sensitive, 90.00% specific and 84.00% accurate with a PPV and NPV of 88.63% and 80.35% , respectively , (Table 3.4 and Figure 3.2).

Table3. 4: ROC analysis shows the sensitivity, specificity and Cut off point for IL-37 and IL-38 according to the Asthma patients.

Metrics		Parameter Values	
		IL-37ng\L	IL-38ng\L
Std. Error		0.045	0.044
P. value		0.001	0.006
Asymptotic 95% Confidence Interval	Lower Bound	0.715	0.893
	Upper Bound	0.750	0.922
Cutoff Point		113.751	55.6100
Area Under Curve (AUC)		80.40%	83.60%
Sensitivity		70.00%	78.00%
Specificity		92.00%	90.00%
Accuracy		80.00%	84.00%
Positive Predictive Value		89.47%	88.63%
Negative Predictive Value		74.19%	80.35%

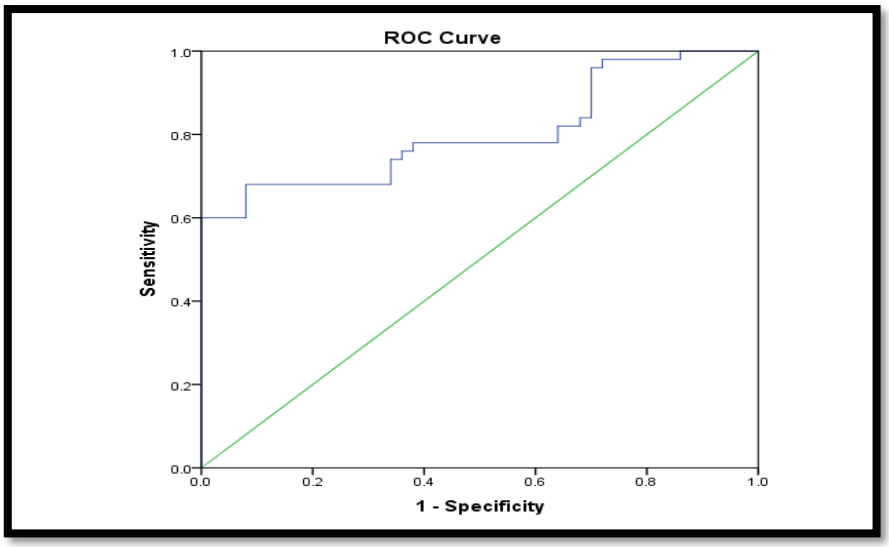


Fig.3.1: ROC Analysis Illustrating The Sensitivity And 1-Specificity Values For IL-37 Of Asthma Patients.

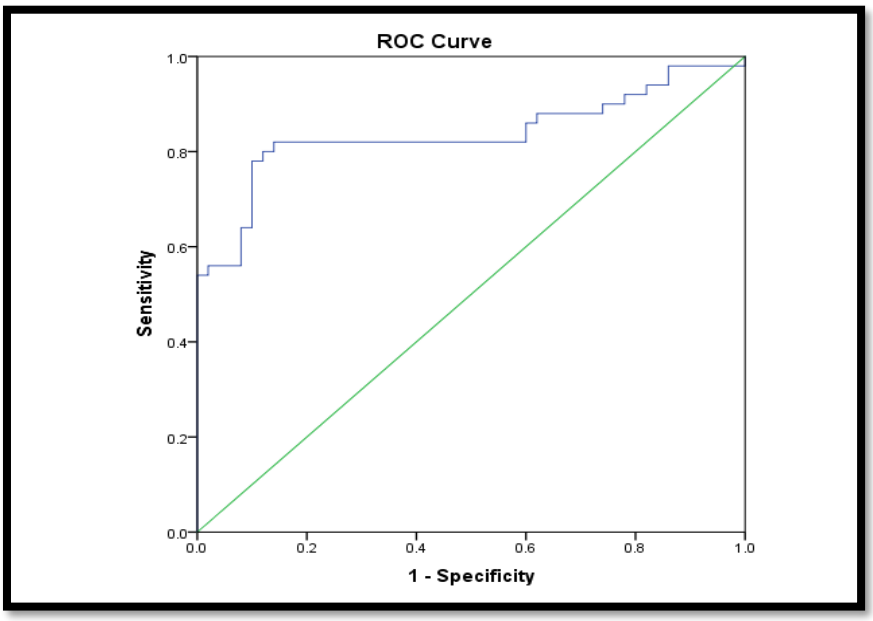


Fig3.2: ROC Analysis Illustrating The Sensitivity And 1-Specificity Values For IL-38 Of Asthma Patients.

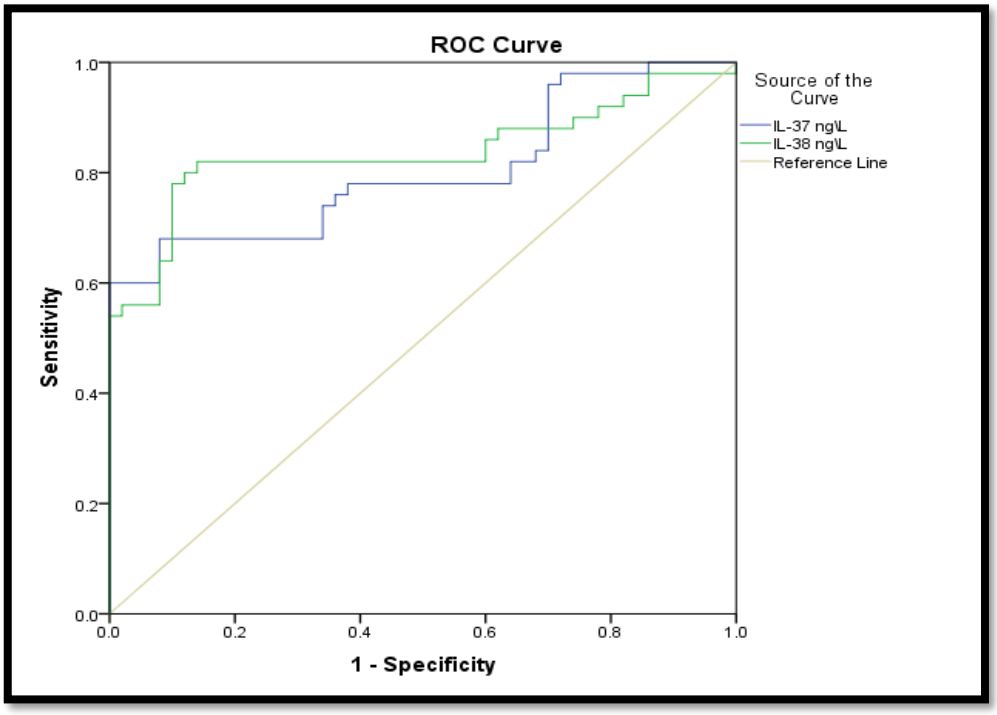


Fig 3 .3: ROC analysis illustrating the sensitivity and 1-specificity values for **IL-37** and **IL-38** of **Asthma** patients .

3.4. Isolation Of Microbiota Of Pediatric Asthma(PA)\ Wheezeres :

Culturing investigation depending on morphological and VITEK compact system results showed that patient group reveals a rate 50(100 %) which includes *S. aureus* that show a high percentage 12 (24.00) % , then *S. Pneumonia* 11(22.00) % . Followed by, *H.influenza* 6 (12.00) % and *K. rosia* 6 (12.00) % and *M.catarrhales* 5 (10.00) % and *S. epidermidis* 4(8.00) % *S. pyogen* 3(6.00) %and , lastly, *P.earoginosa* 1(2.00) % and *S. xylose* 1(2.00) % as shown in(figure 3.4).

Whereas, control group recorded 50(100%), which include was the *S. saprophyticus* most isolated bacteria in this study with percentage 12(25.50) % followed by *S. pyogen* 10(19.60) % then *S. epidermidis* 10 (19.60) % and *S. mutans* 7(13.70) % and *H.influenza* 5(9.80) % and *S. aureus* 3(5.90) % lastly *K. rosia* 2(3.90) % *C.deptheria* 1(2.00) % as shown in (figure 3.5).

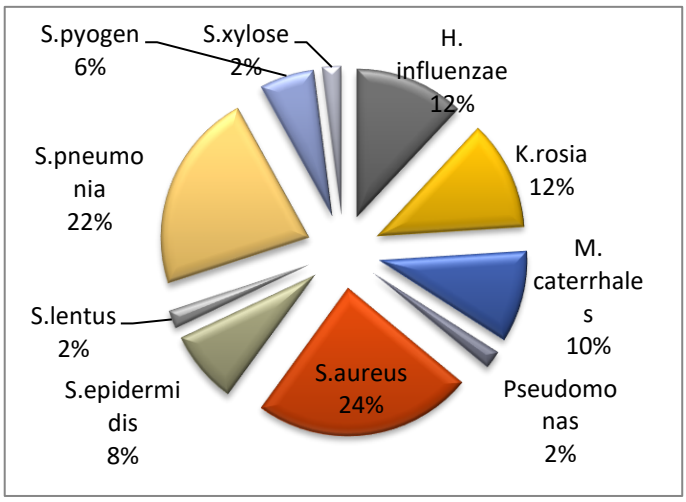


Fig.3.4 Distribution bacterial of patient group

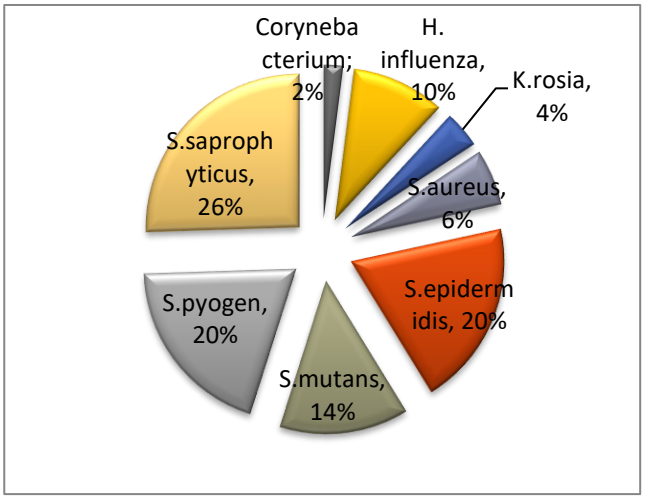


Fig.3.5 Distribution bacterial of control group

3.5: correlation of Bacterial growth with studying group.

The present study findings of bacterial growth showed in tow groups with percentage (100%) , Highly significant differences association was found between bacterial growth in patient and control group such that *M. catarhals , S. aureus , S. mutans , S. pneumonia , S. saprophyticus ,* with significant (0.02 , 0.01 , 0.01 ,< 0.001 ,< 0.001) respectively . On the other hand no significant found in bacterial growth by *C.deptheria, H.influenza, K. rosia , S. epidermidis , S. lentus , S. pyogens, S. xylose in* patient and control, Although having the higher percent of growth .

Table-3.5: Relationship of Bacterial Growth With Studying Group.

Bacteria		Patient	Control	Total	P-value
C.deptheria	No.	1	1		1.00 **ns**
	%	50.0%	50.0%	100.0%	
H.influenza	No.	6	5	11	0.76 ns
	%	54.5%	45.5%	100.0%	
K. rosia	No.	5	2	7	0.26 ns
	%	71.4%	28.6%	100.0%	
M.catarrhales	No.	5	0	5	0.02* sig
	%	100.0%	0.0%	100.0%	
P.earoginosa	No.	1	0	1	0.49 ns
	%	100.0%	0.0%	100.0%	
S. aureus	No.	12	3	15	0.01* sig
	%	80.0%	20.0%	100.0%	
S. epidermidis	No.	4	10	14	0.14 ns
	%	28.6%	71.4%	100.0%	
S. lentus	No.	1	0	1	0.49 ns
	%	100.0%	0.0%	100.0%	
S. mutans	No.	0	7	7	0.01* sig
	%	0.0%	100.0%	100.0%	
S. pneumonia	No.	11	0	11	<0.001* sig
	%	100.0%	0.0%	100.0%	
S. pyogens	No.	3	10	13	0.07 ns
	%	23.1%	76.9%	100.0%	
S. saprophyticu	No.	0	13	13	<0.001*

	%	0.0%	100.0%	100.0%	sig
S. xylose	No.	1	0	1	0.49 ns
	%	100.0%	0.0%	100.0%	
- Chi square test -*. Association is significant at the 0.05 level.					

3.6. association between microbiota and study parameters:

3.6.1.association bacteria species with IL-37 and IL-38 serum level in patient group:

Culturing investigation depending on morphological and VITEK compact system results showed that control group includes, , *C.deptheria, H.influenza,K.rosia, M.catarrhales, P.earoginosa,S. aureus,S.epidermidis,S. pneumonia,S.pyogen, S. xylose , S. lentus*. which revealed Mean for parameter (IL-37) higher in all bacterial species growth on the contrary of Mean of IL-38 in bacterial species growth was lesser .these trouth revealed a positive significant correlation between IL37and all bacterial species growth where (p. value = 0.005). From another point of view, also found relationship in IL-38 and bacterial growth with significant differences (pvalue - 0.006).

Table-3.6 : IL-37 and IL-38 serum level among bacteria species in patient group.

Bacteria		Il-38 ng\l	Il-37 ng\l
C.deptheria	Mean	45.38	84.57
	Std. Deviation	.	.
H.influenza	Mean	74.01	128.58
	Std. Deviation	24.06	63.25
K. rosia	Mean	103.14	221.395
	Std. Deviation	61.04	119.965
M.catarrhales	Mean	96.1139	118.2683
	Std. Deviation	34.43693	57.52463
P.earoginosa	Mean	41.2700	173.7320
	Std. Deviation		
S. aureus	Mean	83.87	164.09
	Std. Deviation	18.53	75.68
S. epidermidis	Mean	89.25	183.44
	Std. Deviation	44.87	92.24
S. pneumonia	Mean	50.50	113.61
	Std. Deviation	26.84	53.51
S. pyogen	Mean	100.21	217.99
	Std. Deviation	32.70	64.26
S. xylose	Mean	78.6240	110.4200
	Std. Deviation		
S. lentus	Mean	44.30	94.45
	Std. Deviation	30.20	64.94
Total	Mean	72.49	150.98
	Std. Deviation	39.23	83.37
LSD		12.91	27.33
P value		Sig 0.006	Sig 0.005
Student T test p-value ≤ 0.05			

3.6.2.association bacteria species with IL-37 and IL-38 serum level in control group:

In same manner in Table 3.7 assess the association between bacterial growth from one side as independent variables and each of IL37and IL38 as dependent variables , Culturing investigation depending on morphological and VITEK compact system results showed that control group includes *C.deptheria, H.influenza ,K.rosia, S.aureus, S.epidermidis, S.mutans, S.pyogen, S.saprophyticus* which revealed Mean for dependent parameter (IL-37) higher in all bacterial species growth on the contrary of Mean of IL-38 in bacterial species growth was lesser . no-significant differences showed between biomarker and bacterial growth in control group**.**

Table-3.7: IL-37 And IL-38 Serum Level Among Bacteria Species In Control Group

Bacteria		IL-37 ng\L	IL-38 ng\L
C.deptheria	Mean	139.1290	31.8760
	Std.Deviation	.	.
H.influenza	Mean	154.1413	76.8540
	Std.Deviation	47.04435	47.07639
K. rosia	Mean	132.9160	69.9726
	Std.Deviation	45.05716	17.89603
S. aureus	Mean	153.2932	83.0344
	Std.Deviation	54.62894	28.72813
S. epidermidis	Mean	199.8370	83.2373
	Std.Deviation	93.40136	45.09955
S.mutans	Mean	162.2129	81.6372
	Std.Deviation	93.49280	42.46577
S. pyogen	Mean	170.1977	76.0637
	Std.Deviation	58.91529	21.97533
S. saprophyticus	Mean	110.4200	78.6240
	Std.Deviation	64.94.	30.20.
Total	Mean	156.9970	80.8412
	N	50	50
	Std.Deviation	67.08634	35.32555
LSD		26.97	12.07

P-value	0.719	0.674
Student T test **p-value ≤ 0.05**		

3.7. bacterial types among asthma severity class in patient group.

Bacterial types after diagnostic according to VITEK2 compact system compare with relationship to asthma severity(mild,modrate,severe) as shown in Table (3.10). The most frequent pathogen isolated from all pediatric groups was *S. aureus* (12 isolates) with predominance in modrate type of asthma severity, although these isolate have ahigher number between them but no-significant relation with severity of asthma (p-value 0.33). followed by *S. pneumonia* (11 isolates) with predominance in modrate type of asthma severity, these isolate have significant relation to severity of asthma (p-value 0.021) . followed by, *H. influenza* (6 isolates) with predominance in mild type of asthma severity, so these isolate have significant relation with types of sevirity (<0.001). *K. rosia* was the fourth microorganism isolated (5 isolates) in this study with predominant in severe types of asthma severity , also these isolate have significant relation with types of severity(<0.001). *M.catarrhales* (5 isolates) found in all types of asthma severity, so these isolate have no-significant relation with types of sevirity (p-value 0.66).

Followed by *S. epidermidis*(4 isolates) with predominance in mild and modrate type of asthma severity but in less count, so these isolate have no-significant relation with types of sevirity (0.41). *S. pyogen* (3 isolates) with predominance in mild type of asthma severity, so these isolate have significant relation with types of severity(p-value 0.02).

C.deptheria, P.earoginosa, S. lentu, S. xylose, least isolated in pediatric pathogen were isolated from pediatric group (1,1,1,1 respectively isolate) so these isolate have no- significant relation with types of sevirity (p- value 0.30, 0.39, 0.39, 0.39 respectively).

Table3. 8. Bacterial Species Among Asthma Severity Class In Patient Group.

Bacteria		Severity			Total	P- value
		Mild	Moderate	Severe		
C.deptheria	No.	1	0	0	1	0.30
	%	100.0%	0.0%	0.0%	100.0%	
H.influenza.	No.	5	1	0	6	<0.001
	%	83.3%	16.7%	0.0%	100.0%	
K. rosia	No.	0	0	5	5	<0.001
	%	0.0%	0.0%	100.0%	100.0%	
M.catarrhales	No.	1	2	2	5	0.66
	%	20.0%	40.0%	40.0%	100.0%	
P.earoginosa	No.	0	1	0	1	0.39
	%	0.0%	100.0%	0.0%	100.0%	
S. aureus	No.	2	8	2	12	0.33
	%	16.7%	66.7%	16.7%	100.0%	
S. epidermidis	No.	2	2	0	4	0.41
	%	50.0%	50.0%	0.0%	100.0%	
S. lentus	No.	0	1	0	1	0.39
	%	0.0%	100.0%	0.0%	100.0%	
S. pneumonia	No.	1	7	3	11	0.021
	%	9.1%	63.6%	27.3%	100.0%	
S. pyogen	No.	3	0	0	3	0.02
	%	100.0%	0.0%	0.0%	100.0%	
S. xylose	No.	0	1	0	1	0.39
	%	0.0%	100.0%	0.0%	100.0%	
Total	No.	15	23	12	50	<0.001
	%	30.0%	46.0%	24.0%	100.0%	

- Chi square test
-*. Association is significant at the 0.05 level.

3.8. association Between Bacterial Species and Demographic Characteristics

The present study findings of bacterial growth showed that both gender have bacterial growth with level (female 41.6% and male 58.4%) . some bacterial showed significant differences between studied groups according to the gender . *S. aureus, S. mutans, S. pneumonia,* have significant differences *(p-value* 0.04, 0.02, 0.02 recpectively). So that all other bacterial growth have non-significant differences according to the gender when compared between the studied groups.

However, according to the age, study findings of bacterial growth showed that all age groups have bacterial growth with age level of (1-5) years in more significant differences with *S. saprophyticus and S. pneumonia (p-value* 0.01, 0.01 respectively) .that revealed these bacteria highly connected with thses individual in that age(more prominent in 4 years old) , also another bacterial growth appear with these age but non-significant differences *C.deptheria, H.influenza, K. rosia, M.catarrhales, P.earoginosa, S. aureus, S. epidermidis, S. lentus, S. mutans, , S. pyogen, S. xylose.(p-value* 0.29, 0.93, 0.30, 0.17, 0.69, 0.43, 0.70, 0.74, 0.36, 0.53, 0.74.)

According to the season, the current study showed that all bacterial collection highly growth in winter and autumn but lesser in summer. *S.aureus* and *S. pneumonia* revealed in more growth with autumn and winter but lesser in summer so these normal flora have significant differences when comparetion with other normal flora in these season (p-value 0.06 , 0.05 respectively), also *S. mutans showed* highly growth in

winter but no growth in summer and autumn with significant differences (p-value 0.02), also *S. saprophyticus* have highly growth in winter but less in autumn and no growth in summer so these normal flora have significant differences in connection to these sesason (p-value 0.03) . as showed in table (3.10)

Table3. 9 . Bacterial Distribution According To Sex , Age Group And Season.

Bacteria		Gender				Age (in year)							Season				
		Female	Male	Total	p-value	1-2	2.5-3	3.5-4	4.5-5	5	Total	P-value	Summer	Winter	Autumn	Total	P-Value
C.deptheria	No.	2	0	2	0.17	0	0	2	0	0	2	0.29	0	1	1	2	0.97
	%	100.0%	0.0%	100.0%		0.0%	0.0%	100.0%	0.0%	0.0%	100.0%		0.0%	50.0%	50.0%	100.0%	
H.influenza	No.	6	5	11	0.51	0	1	2	3	5	11	0.93	0	5	6	11	0.77
	%	54.5%	45.5%	100.0%		0.0%	9.1%	18.2%	27.3%	36.4%	100.0%		0.0%	45.5%	54.5%	100.0%	
K. rosia	No.	2	5	7	0.69	0	1	0	1	4	7	0.30	0	2	5	7	0.38
	%	28.6%	71.4%	100.0%		0.0%	14.3%	0.0%	14.3%	57.1%	100.0%		0.0%	28.6%	71.4%	100.0%	
M.catarrhales	No.	1	4	5	0.15	1	1	2	1	0	5	0.17	0	2	3	5	0.80
	%	20.0%	80.0%	100.0%		20.0%	20.0%	40.0%	20.0%	0.0%	100.0%		0.0%	40.0%	60.0%	100.0%	
P.earoginosa	No.	0	1	1	1.00	0	0	1	0	0	1	0.69	0	0	1	1	0.56
	%	0.0%	100.0%	100.0%		0.0%	0.0%	100.0%	0.0%	0.0%	100.0%		0.0%	0.0%	100.0%	100.0%	
S. aureus	No.	3	12	15	0.04	0	3	5	1	4	14	0.43	1	4	10	15	0.06
	%	20.0%	80.0%	100.0%		0.0%	21.4%	35.7%	7.1%	28.6%	100.0%		6.7%	26.7%	66.7%	100.0%	

S. epidermidis	**No.**	**6**	**8**	**14**	**1.00**	**0**	**1**	**4**	**3**	**4**	**12**	**0.70**	**0**	**8**	**6**	**14**	**0.79**
	%	**42.9%**	**57.1%**	**100.0%**		**0.0%**	**7.1%**	**28.6%**	**21.4%**	**28.6%**	**100.0%**		**0.0%**	**57.1%**	**42.9%**	**100.0%**	
S. lentus	**No.**	**1**	**0**	**1**	**0.42**	**0**	**0**	**0**	**0**	**1**	**1**	**0.74**	**0**	**1**	**0**	**1**	**0.62**
	%	**100.0%**	**0.0%**	**100.0%**		**0.0%**	**0.0%**	**0.0%**	**0.0%**	**100.0%**	**100.0%**		**0.0%**	**100.0%**	**0.0%**	**100.0%**	
S. mutans	**No.**	**6**	**1**	**7**	**0.02**	**0**	**2**	**3**	**2**	**0**	**7**	**0.36**	**0**	**7**	**0**	**7**	**0.02**
	%	**85.7%**	**14.3%**	**100.0%**		**0.0%**	**28.6%**	**42.9%**	**28.6%**	**0.0%**	**100.0%**		**0.0%**	**100.0%**	**0.0%**	**100.0%**	
S. pneumonia	**No.**	**1**	**10**	**11**	**0.02**	**0**	**1**	**1**	**8**	**1**	**11**	**0.01**	**1**	**3**	**7**	**11**	**0.05**
	%	**9.1%**	**90.9%**	**100.0%**		**0.0%**	**9.1%**	**9.1%**	**72.7%**	**9.1%**	**100.0%**		**9.1%**	**27.3%**	**63.6%**	**100.0%**	
S. pyogen	**No.**	**6**	**6**	**12**	**0.76**	**0**	**1**	**5**	**2**	**5**	**13**	**0.53**	**0**	**8**	**5**	**13**	**0.67**
	%	**46.2%**	**53.8%**	**100.0%**		**0.0%**	**7.7%**	**38.5%**	**15.4%**	**38.5%**	**100.0%**		**0.0%**	**61.5%**	**38.5%**	**100.0%**	
S. saprophyticus	**No.**	**8**	**5**	**13**	**0.14**	**2**	**1**	**0**	**13**	**3**	**19**	**0.01**	**0**	**10**	**2**	**12**	**0.03**
	%	**61.5%**	**38.5%**	**100.0%**		**15.4%**	**7.7%**	**0.0%**	**46.2%**	**23.1%**	**100.0%**		**0.0%**	**84.6%**	**15.4%**	**100.0%**	
S. xylose	**No.**	**0**	**1**	**1**	**1.00**	**0**	**0**	**0**	**0**	**1**	**1**	**0.74**	**0**	**0**	**1**	**1**	**0.56**
	%	**0.0%**	**100.0%**	**100.0%**		**0.0%**	**0.0%**	**0.0%**	**0.0%**	**100.0%**	**100.0%**		**0.0%**	**0.0%**	**100.0%**	**100.0%**	
Total	**No.**	**42**	**58**	**100**	**0.00**	**3**	**12**	**25**	**33**	**27**	**100**	**0.23**	**2**	**51**	**47**	**100**	**0.22**
	%	**41.6%**	**58.4%**	**100.0%**		**3.0%**	**12.0%**	**25.0%**	**33.0%**	**27.0%**	**100.0%**		**2.0%**	**51.5%**	**46.5%**	**100.0%**	
Chi square test **Association is significant at levels 0.05**																	

3.9. Typing Of Bacterial Sample According To Gram Stain

In present study depended on gram staining for typing bacterial samples to two groups , gram positive (have purple colour) and gram negative(have red colour) by retained or without retained gram dye .

In table 11, revealed distribution gram positive and negative between patient and control where percentage of gram negative in all studying group less than gram positive (17.0% gram negative , 83.0% gram positive) . also finding significant differences between two groups (p-value 0.042). **figure 3.6** also illustrate that percentage bacterial negative less than bacterial positive .

Table 3.10. Typing of bacterial isolate according to gram stain with studying group .

Variable	Level	Control		Patient		Total		P-Value
		Number	Percentage %	Number	Percentage %	Number	Percentage %	
Bacteria Type	Gram Negative	5	10.0%	12	24.0%	17	17.0%	0.042
	Gram Positive	45	90.0%	38	76.0%	83	83.0%	
-The chi-square test has been utilized to analyse the categorical variables.								
-*. Association is significant at the 0.05 level.								

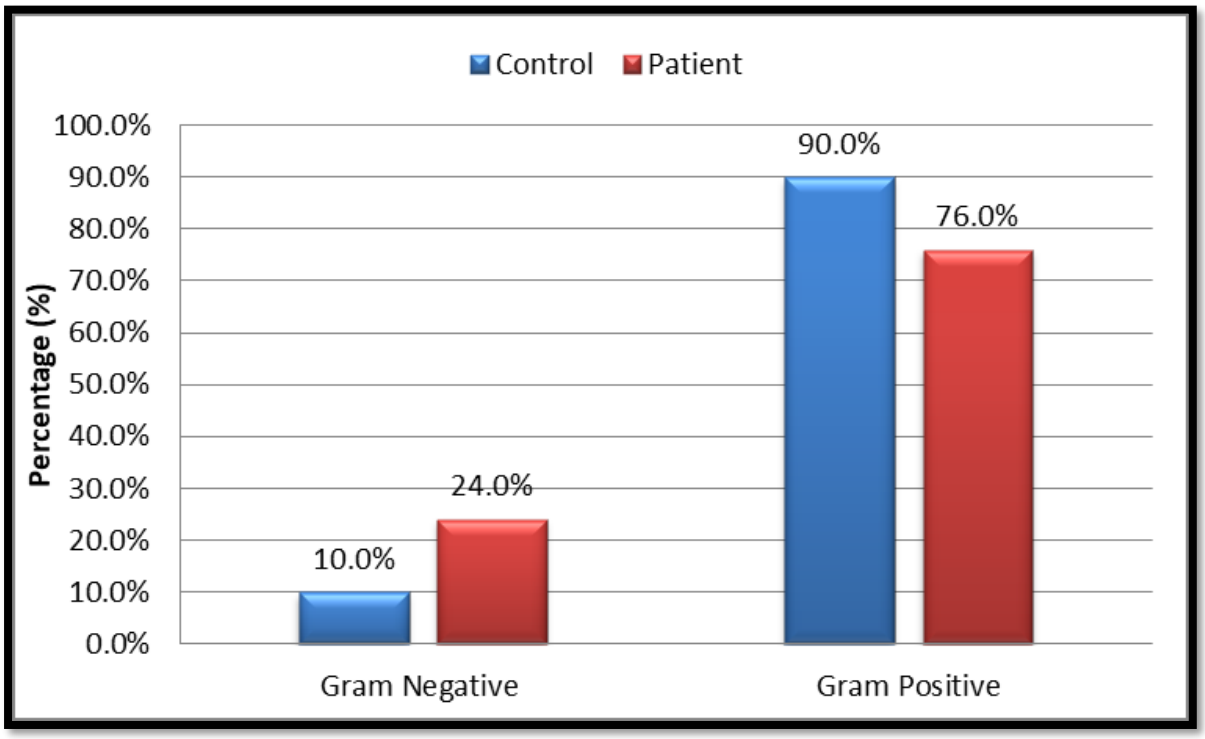


Figure 3. 6: Bacteria Type Distribution and Characteristics of Patients in comparison to the Control group.

3.10 distribution studying group in relation to bacterial type

in current study after typing bacterial sample with gram stain into gram positive and gram negative ,now asses the relationship distribution between studying group and bacterial typing (gram positive , gram negative) .

where founding in patient , gram positive bacteria abundance than gram negative with trending for *S.aureus* , *S.Pneumonia* , *K.rosia* (31.6% , 28.9% , 13.2% respectively) . while gram negative bacteria have trending with Heamophilus , Moraxella (50.0% , 41.7% respectively) . so these groups have highly significant (p-value 0.002**) **figure 7.** on other hand in control groups have gram positive bacteria also highly

abundance than gram negative with regared to *S. Pyogen* , *S.epidermidis* , *S.Saprophyticus* more trending in this group (22.2% , 20.0% , 28.9% respectively) .while *H.influenza* was only gram negative bacteria in control group (100.0%). So these group have highly significant differences (p-value 0.001**) **figure 8.**

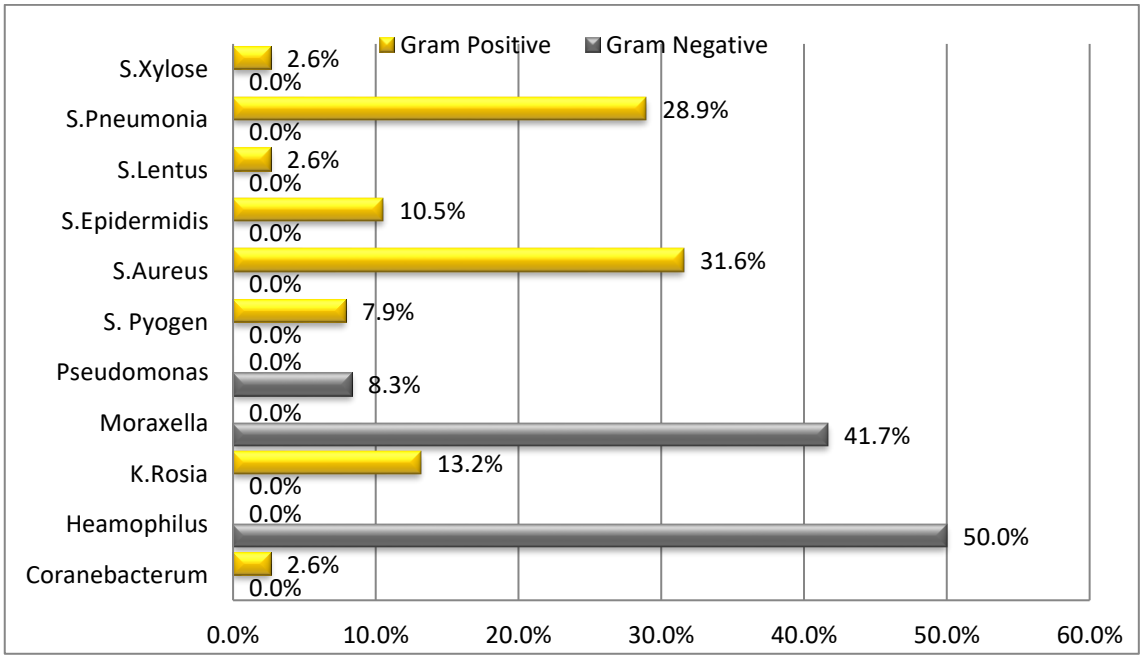


Figure 3.7: Distribution and Characteristics of the Patients Group According to the Bacteria Type.

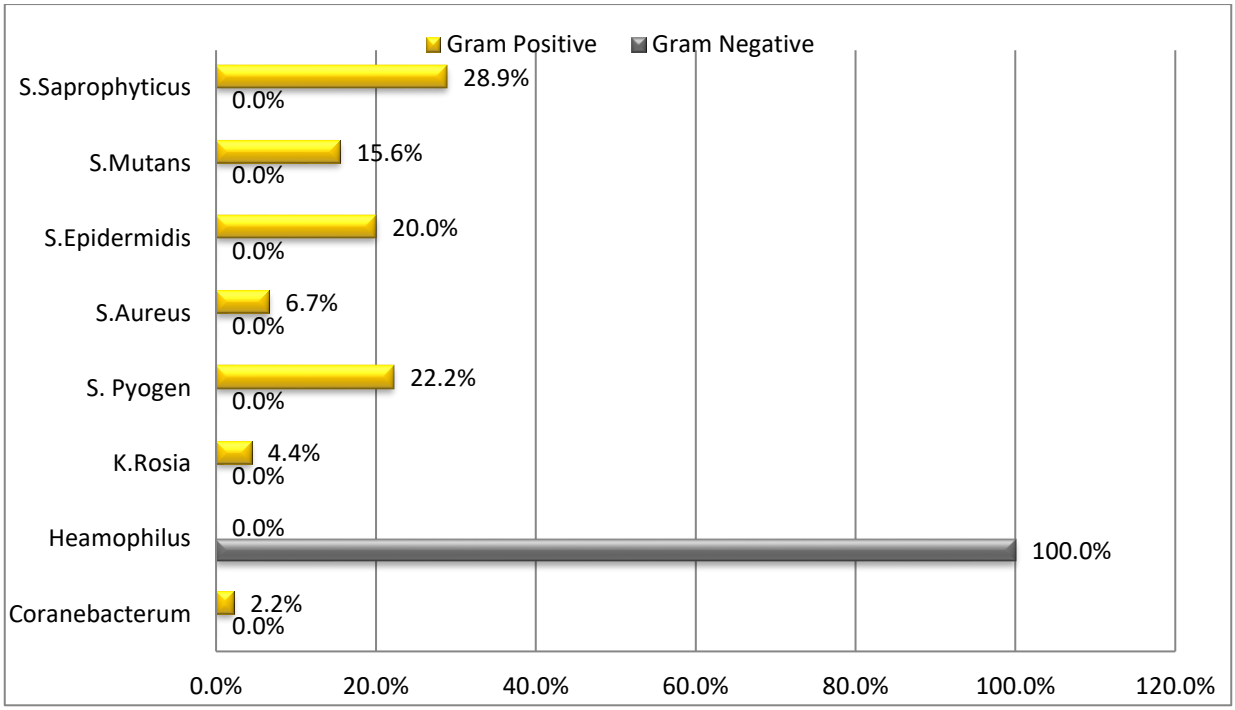


Figure3. 8: Distribution and Characteristics of the Control Group According to the Bacteria Type

3.11 Distribution Bacterial Swabs Between Studying Group In Relation To Number Of Isolate .

3.11.1 Single Or Multiple Bacterial Isolate Of Patient Group.

The laboratory method used to collecting the sampling for patient group we discover that swabs containing double bacterial type have the largest number (28) between all culturing swabs with percentage (56 %) , flowed by swabs that have single bacterial type (may be from the same genus or different) have number (20) with percentage(40 %), lastely the swabs that have trible bacterial type (may be from the same genus or different) was just view number (2) with percentage (4 %), so no swabs have quadrate bacterial sample showed during sampling collecting .

Table 3.11 : **single or multiple bacterial isolate of patient group.**

Patient			
bacterial contining in swabs	**Swabs number**	**Gram Positive \negative**	**Percentage 100%**
single	**20**	**All positive,**	**40 %**
double	**28**	**Positive + negative**	**56 %**
		Positive + positive	
Trible	**2**	**2 positive + 1 negative**	**4 %**
		All trible positive	
total	**50**		***100%***

3.11.2 Single Or Multiple Bacterial Isolate Of Control Group.

In same manner in patient group , The laboratory method used to collecting the sampling for control group we discover that swabs containing single bacterial type have the largest number (29) between all culturing swabs with percentage (58 %) , flowed by swabs that have double bacterial type (may be from the same genus or different) have number (18) with percentage(36 %), lastely the swabs that have trible bacterial type (may be from the same species or different) was just view number (3) with percentage (6 %), so no swabs have quadrate bacterial sample showed during sampling collecting.

Table 3. 12 : Single Or Multiple Bacterial Isolate Of Control Group.

control			
bacterial contining in swabs	**Swabs number**	**Gram Positive \negative**	**Percentage %**
single	**29**	**All positive,**	**58 %**
Double	**18**	**Positive + negative**	**36 %**
		Positive + positive	
Triple	**3**	**2 positive + 1 negative**	**6 %**
		All trible positive	
total	**50**		**100%**

Chapter Four

Discussion

4.1. Demographic Characteristics Of Study Groups:

The present investigation discovered that while both genders (0–5 years) were dominating in pediatric asthma, the differences were statistically insignificant. These results did not align with those of other recent studies. According to (Loewenthal *et al.,* 2024) , The explanation is that women are more prone than men to acquire asthma, and when they do, it's usually more severe. Numerous internal and external factors play a complex role in the relationships between sex and asthma .

In agreement with the present investigation, a previous study showed that gender disparities among the four groups remained non-significant (p-value = 0.4128) (Alzaatreh, Al Bataineh, and Salman, 2023).

The associations between clinical phenotypes (disease severity, temporal symptom pattern), type of inflammation, environmental triggers (e.g., viruses, allergens, pollutants), response to treatment, and comorbidities are unique to pediatric wheezing disorders and are age-dependent and interactive (Pijnenburg *et al.,* 2022).

According to the resident, the study's own findings showed that rural patients' asthma was significantly worse because of their way of life and increased exposure to air pollution from traffic. These findings align with the findings of (Li, Jahan, and Newcomb, 2023). Accept the current study's findings, as noted the factors that were present when participants residing in rural areas were colonized. (*Corynebacterium, Sphingomonas, Moraxella, and Alloiococcus*) compared to those from the urban environment (*Sphingomonas, Moraxella, and Staphylococcus*), which were linked to higher exposure to environmental pollutants (Ahmed *et al.,* 2019). An additional study concurs by revealing Those who lived in cities, had no history of infectious diseases as children, and had between 0 and 5

years between the diagnosis of asthma and menarche all continued to show a strong correlation (Juber and others, 2023).

Current study discovered a connection between the following risk factors: season, ICS use, eczema, allergic rhinitis/conjunctivitis, air quality, and microbiota composition during periods of severe exacerbation. In our study, all of these findings were significantly correlated with the severity of pediatric asthma. Additionally, we concur with a study that demonstrated little variation in the respiratory microbiota between children with mild/moderate and those with severe asthma (Eriksen *et al.,* 2022). Further research is therefore warranted to understand the potential role of this bacterium in asthma (exacerbation) prevention.

Several other environmental and lifestyle factors, including exposure to cigarette smoke, having an animal in the home, passive smoking, having a history of asthma, and eating an unhealthy diet, have been shown to significantly contribute to the development of pediatric asthma. Our research in this section has corroborated a recent study that explains how these factors, along with respiratory infections and an unhealthy diet, also involve mechanisms that are only partially understood and involve oxidative stress and pro-inflammatory triggers. Their influence on the onset and severity of pediatric asthma is a crucial issue that merits extensive investigation (Vincenzo *et al.,* 2023).

4.2. Laboratory Parameters Of The Studied Group:

4.2.1. lab parameters distribution between patient and control.

The lab parameter level reported in both patient and control, mean was higher in patients other than control in all parameters .

There were notable differences in IL-37 between the groups under study (0.029) and IL-38 between the groups under study (0.026). Therefore, all blood parameters (eosinophil, lymphocyte and neutrophil) exhibit

extremely significant values with the groups under study (p-value <0.001, <0.001, and 0.023, respectively). The average number of patients had elevated serum total IgE levels (626.5136) compared to normal limits (100-200IU/Ml in children).

In the current investigation, we discovered that the children with asthma had significantly higher serum levels of IL-38 than the healthy control patients. Furthermore, only in the atopic group did the serum levels of IL-38 show an inverse correlation with the degree of eosinophilia, indicating that IL-38 serum levels may serve as a biomarker for children patients with atopic asthma (Kamal *et al.*, 2022).

Children with asthma had significantly higher serum concentrations of IL-38 than control patients, regardless of steroid treatment. This rise was comparable to that of periostin, IL-5, IL-6, IL-13, IL-17, IFN-γ, and IL-1β.The findings of these reports are consistent with our investigation, which found that IL-38 levels were higher in patients than in controls (de Graaf *et al.*, 2022).

IL-38 may be a potential cytokine in the management of allergic disorders, according to a study by (Tsang, Sun, and Wong, 2020) on the explanation of IL-1 family cytokines in the development and pathogenesis of allergic diseases.

Recombinant versions of IL38 may be employed as a therapeutic for the treatment of immunological or inflammatory illnesses, according to findings from such studies that reveal low circulation levels of IL-38 in some clinical circumstances indicate a relative "deficiency" of IL-38 (de Graaf *et al.*, 2022).

Similarly, it was discovered that IL-37 had a strong significant relationship with asthmatic patients, indicating that these findings are consistent with previous studies.The degree of IL-37 expression varies amongst illnesses.

Since IL-37 expression in these conditions is mostly high and positively correlated with disease activity, the prospective use of IL-37 as a novel therapeutic target may be advantageous in the control of the inflammatory, metabolic, and immunological response as well as the development of cancer (Tao and Su, 2021).

In contrast to a previous study, the current study found that IL-37 was reduced in asthma, indicating that IL-37's action is reduce the inflammation and remodeling of the airways, suppress PYCARD, S100A9, and CAMP gene transcription, which are associated with allergic inflammation (Lv *et al.*, 2018. Zhu *et al.*, 2018; Huang *et al.*, 2018; Meng *et al.*, 2019). Additionally, it has been demonstrated in other studies that asthma is linked to an increased risk of lung cancer; this information suggests that IL-37 may be an inhibitor of lung cancer (Kantor *et al.*, 2019).

The rationale behind varying IL-37 levels that Since its development, the cytokine has been shown to have broad protective benefits against cancers, autoimmune illnesses, metabolic diseases, allergy diseases, and infectious diseases. Immune and certain non-immune cells were the primary producers of IL-37 in response to an inflammatory stimuli , The immunoregulatory role of IL-37 has been steadily elucidated and demonstrated to inhibit both innate and adaptive immunity (Gu *et al.*, 2023). An immunosuppressor with anti-inflammatory properties in inflammatory illnesses is interleukin-37 (Nold *et al.*, 2010).

Noted our findings include numerous results from previous and recent studies, such as the following: Patients with atopic asthma frequently have a high eosinophilic count, and the level of eosinophilia is usually correlated with the degree of asthma severity and is considered as an indication of asthma exacerbation (Koh and Choi, 2002 and Tran *et al.*, 2014). In our report, blood parameters (eosinophil, neutrophil, lymphocyte) were highly

in all children with asthma \ exacerbation other than health child. In contrast to those with less severe asthma, those with severe asthma exhibited a larger prevalence of atopy (64% vs. 50%), with 18.4% and 46.9% of cases showing neutrophilic and eosinophilic inflammation, respectively. High peripheral blood and airway eosinophilic levels are observed in patients with eosinophilic asthma. Eosinophils build up in the lungs and bronchial passages, where they release cytokines and cytotoxic mediators. This exacerbates the severity of asthma by causing inflammatory reactions and tissue damage (Druilhe, Letuve, and Pretolani, 2000)and (Trivedi *et al.*, 2007). discovered these outcomes along with our findings.

4.2.2. Correlation Laboratory Parameter Among Asthma Severity Class In Patient Group:

where the patient group's IL-37 and IL-38 levels varied significantly depending on the asthma severity class. In contrast, mean IL-38 levels were lower than mean IL-37 in the asthma class (80.63, 85.01, 73.11), even though mean IL-37 levels increase in mild, moderate, and severe asthma (mean 172.31, 159.05, and 134.08, respectively). This our discovered similarity to another result in such study . According to current theory, IL-37 expression only rises in highly inflammatory states in order to prevent immunological storms and inflammatory intensification; it does not rise in non-inflammatory or mildly inflammatory circumstances (Jia, Liu, and Han, 2018). Serum interleukin 38 (IL-38) levels are raised in a number of inflammatory and autoimmune disorders in a similar way (Kamal *et al.*, 2022). In contrast, such study investigation revealed that there was no statistically significant difference in the levels of IL-37 in asthmatics classified as mild (48.15 ± 8.67 pg/ml) or moderate (48.30 ± 8.53 pg/ml; P = 0.95) (Charrad *et al.*, 2016).

In individuals with pediatric asthma, Chu and his colleagues' (Chu *et al.*, 2016) findings indicate a significant rise in IL-38 serum levels. A recent study also suggested that IL-38 serum levels in pediatric patients may serve as a biomarker for atopic asthma (Kamal and others, 2022). Comparable in anti-inflammatory potency to IL-37 and IL-36Ra, IL-38 functions best at low quantities and may have the opposite effect at larger concentrations.As of (Xie et *al.*, 2019).

Individuals with eosinophilic asthma had significantly lower serum IL-38 levels than individuals without eosinophilic asthma (p=0.011) (Kamal *et al.*, 2022) . IL-38's up- or down-regulation may therefore be explained by the type of cell and condition under investigation. In vitro, keratinocytes that express high levels of endogenous IL-38 are inhibited by IL-17, IL-22, IFN-γ, and IL-36γ (Mercurio *et al.*, 2018).

When peripheral blood mononuclear cells (PBMCs) are at rest, they exhibit modest levels of endogenous IL-38 expression, which can be stimulated by IL-17, TNF, and IFN-γ. (Kim *et al.*, 2016)

Though puzzling, this differential control of IL-38 by these innate inflammatory cytokines is explicable. In order to preserve homeostasis, the skin barrier may naturally benefit from IL-38's ongoing anti-inflammatory presence (Lachner *et al.*, 2017).

The justification is that the study of chemokines and cytokines is fast developing in the fields of allergy, inflammation, and laboratory medicine. Dysregulation of cytokines and chemokines, including T helper (Th) type 1 and 2 cytokine imbalance, Th17 cell over-activation, aberrant regulatory T/B cells and regulatory cytokines, and cytokine/chemokine storm, is commonly linked to inflammatory disorders. Mild patients had a slightly greater levels of IL-38 than severe patients (Sun *et al.*, 2023)

4.3. Diagnostic Utility Of Study Parameters:

This analysis might possibly reflect that higher level of IL-37 and IL-38 in asthmatic individual compared to control group gives the possibility of using of these parameters as a marker in diagnosis of asthma . In the IL-1 family, IL-38 is an anti-inflammatory cytokine that shares characteristics with IL-37, another anti-inflammatory cytokine (Cavalli*et al.,* 2018).

The current investigation revealed Upon analyzing the ROC curve for IL-37 in the patient group, it was found that the area under the curve (AUC) was 80.40%. This indicates a larger prediction value at an optimal cutoff point of IL-37 = 113.751, resulting in high sensitivity, specificity, and accuracy of 70.00%, 92.00%, and 80.00%, respectively. The positive predictive value (PPV) was identified as 89.47%, while the negative predictive value (NPV) was 74.19%.

The IL-1 family has a recently identified member called interleukin-37 (IL-37). Since its development, the cytokine has been shown to have broad protective benefits against cancers, autoimmune illnesses, metabolic diseases, allergy diseases, and infectious diseases. In response to an inflammatory stimuli, immune and certain non-immune cells released the majority of IL-37(Gu *et al.*, 2023). showed up Numerous studies that match our findings imply that IL-37 might be essential for the etiology of asthma (Imaeda *et al.,* 2013). Another study found that in both macrophagic and epithelial cells, elevated levels of IL37 (whether external or intracellular is unknown) are correlated with a reduced response to inflammatory stimulation (Boraschi *et al.,* 2011).

The validity criteria for IL-38 were similar to IL-37. It was 78.00% sensitive, 90.00% specific, and 84.00% accurate at an AUC of 83.60% at a cutoff point of IL-38= 55.6100, With a PPV of 88.63% and NPV of 80.35% . (Figure 3.2 and Table 3.4).

IL-38 may prove to be a valuable biomarker for both prognosis and treatment of atopic asthma in young patients , In the such investigation, there was a significant difference ($p < 0.001$) in blood IL-38 levels between children patients with bronchial asthma and healthy controls (Kamal *et al.,* 2022).

IL-38 may be a potential cytokine in the management of allergy disorders, according to a recent review by (Tsang, Sun, and Wong, 2020) on the significance of IL-1 family cytokines in the development and pathogenesis of allergic diseases.

4.4. Isolation of microbiota of pediatric asthma(PA)\ wheezeres :

Based on morphological and VITEK compact system results, the culture research revealed that the patient group has a rate of 50(100%), with *S. aureus* showing the highest proportion at 12(24.00)%, followed by *S. pneumoniae* at 11(22.00)%. Next in order of percentage are *H.influenza* 6 (12.00)%, *K. rosia* 6 (12.00)%, *M.catarrhales* 5 (10.00)%, and *S. epidermidis* 4 (8.00)%. *S. xylose* 1(2.00) % and *P.earoginosa* 1(2.00) % are the final two. *S. pyogen* 3(6.00) % (table 3.5 and figure 3.3) . In contrast, the control group recorded 50(100%), of which the majority of isolated bacteria in this study, *S. saprophyticus*, made up percentages of 12(25.50)%, *S. pyogen* 10(19.60)%, *S. mutans* 7(13.70)%, *H.influenza* 5(9.80)%, *S. aureus* 3(5.90)%, *K. rosia* 2(3.90)%, and *C.deptheria* 1(2.00)%, .

Using normal laboratory techniques, nasopharyngeal swabs were taken from asthmatic and healthy control subjects, revealing a wide range of microbiota variations between the research groups. Each of the children with asthma and the healthy children under study appeared to have a different range of nasal microbiome composition. This is consistent with research by (Teo S. M. *et al.,* 2015 and M.-L. von Linstow *et al.,* 2013) that

indicates dysbiosis of the microbiome raises the risk and severity of asthma. Asthma and Caesarean sections have a well-known and infamous relationship , It has been demonstrated that the more physiological vaginal microbiome does not colonize women born by caesarean section (Stokholm .J *et al.*, 2016).

However, we were unable to identify a particular microbiome profile, with the exception of the combination of *S. aureus* and *S. pneumoniae*, which was found to be more prevalent in individuals with asthma (22.0 %, 24.0 %). According to multiple research (G. Biesbroek *et al.*, 2014 and L. P. Schenck *et al.*, 2016), the presence of *S. pneumoniae* (either with *H. influenzae* or *S. aureus*) in the nasopharyngeal microbiome is a predictor for wheeze in babies, these result according with current study.

Among the healthy controls, the highest percentage of coagulase negative staphylococci (CoNS) was found (25.50% and 19.50%, compared to 8.00% and 0% in asthmatics).According to another study, the majority of the healthy controls (90% vs. 3.33% in asthmatics, p=0.009) had this assessment. In healthy patients, CoNS are typically detected on the skin, mucosa, and nasal flora (K. Becker *et al.*, 2014; H. B. Allen *et al.*, 2014) , these result similarity to current investigation.

The rationale behind these variations in microbiota, Although the underlying origins of asthma are still unknown, a dysbiotic microbial ecology in the gut may account for at least some of the disease's primary mechanisms, which include loss of epithelial integrity and an overreactive immune response. It is obvious that dysbiosis has a significant role in the etiology, severity, and persistence of asthma, regardless of whether it is the only cause or one of several contributing factors. Before starting therapeutic trials that target gut microbiota with the goal of preventing and

treating asthma, a more complete and in-depth understanding of the function of bacteria in the disease is needed In(Salameh *et al.,* 2020)

A number of external factors, such as food, antibiotic use, or smoke exposure, can cause dysbiosis of the gut microbiota, which has been linked to alterations in both local and systemic immune responses, including lung inflammation (Budden *et al.,* 2017) and (Hufnagl *et al.,* 2020).

4.5. Relationship Of Bacterial Growth With Studying Group.

The current investigation provide a detailed prospective characterization of bacterial communities within the human NP microbiome during the first years of life . The NP microbiome was dominated by seven common genera: *Haemophilus, Streptococcus, Moraxella,* Staphylococcus, *Pseudom onas, and Corynebacterium ,Kocuria*. This is consistent with previous studies of NP microbiome composition in children aged 12–14 months (Biesbroek *et al.,* 2014, Bogaert *et al.,* 2011) .

At this age, the microbial makeup of the URT varied greatly, with the most common phyla in the URT among children with asthma being Firmicutes (*Staphylococcus,Streptococcus*) and Proteobacteria (*Moraxella, Haemophilus, Neisseria*). The likelihood of developing wheeze at age 5 was linked to *Streptococcus* nasopharyngeal colonization during the first seven weeks of life, according to a pooled analysis of two investigations.According to (Lee *et al.,* 2019 and Durack *et al.,* 2018), this result is consistent with ongoing research. The conclusion Our result was consistent with previous research that found that in early-sensitized children, asymptomatic colonization of *Streptococcus, Haemophilus,* and *Moraxella* in the URT enhanced the risk of chronic wheeze at age five ,

Infants with early *Streptococcus* colonization also exhibited atopic behavior by the age of two and persistent wheezing by the age of five (Teo SM *et al.,* 2015). Thus, in preschoolers with atopic conditions, early

colonization of *Streptococcus* in the URT may predict the development of wheezing or asthma. When wheeze was defined at the 7-year mark (Toivonen L *et al.,* 2020) .

Early in life, there have been reports of high rates of *S. aureus* nasal colonization (Bisgaard et al., 2007, Bisgaard *et al.*, 2010). A recent study in African children found that *S. aureus* was colonizing 42% of infants at 1 month and 12% at 12 months of age, This trend was mirrored in maternal colonization rates (Schaumburg *et al.,* 2014) and is remarkably similar to the patterns we observed (80.0% for patient at 1-5 year, 20.0% for control at the same age). Few studies have looked at longitudinal colonization, however. According to one study, nasopharyngeal *S. pneumoniae* is linked to acutely worsened wheezing throughout the first three years of life (Teo *et al.,* 2018), hence our investigation evaluated this conclusion.

Children with asthma had higher levels of *K. rosea*, both by itself and in combination with other organisms, than did healthy controls; nevertheless, the difference was not statistically significant (p=0.26). It was discovered that people with more severe asthma experienced it more prominently. Gram-positive, catalase-positive, and oxidatively positive cocci are known as Kocuria rosea. Malachite green and other commercial colors are reported to be biodegradable by Kocuria rosea (G. K. Parshetti *et al.*, 2012 and G. K. Parshetti *et al.,* 2010).

Children who had a nasal microbiota dominated by *Haemophilus* and *Moraxella* during a stable illness were more likely to experience recurrent exacerbations of their asthma due to infection symptoms (McCauley *et al.,* 2022).

In past study for *Moraxella* included a representation of *M. catarrhalis*, an unencapsulated, human-restricted Gram-negative bacterium that has been linked to pathogenicity in the inner ear and respiratory system as well as commensal NP colonization (de Vries *et al.,* 2009).

The aforementioned results align with the established capacity of *Moraxella* to generate biofilms (de Vries *et al.*, 2009), which provide resistance against antibiotics and facilitate the co-colonization of common bacteria like *S. pneumoniae* and *H. influenzae* (Verhaegh *et al.*, 2011). *Haemophilus* was nearly only detected in 54.5% of patients and 45.5% of health control cases, indicating that it can linger in the nasopharynx for a considerable amount of time.

In comparison to healthy controls, wheezing children have increased levels of *Haemophilus* and *Moraxella* as well as elevated *Neisseria* , The entire upper respiratory system has shown these correlations (Bisgaard *et al.*, 2010).

Our analysis revealed that *C.deptheria* were regularly linked to healthy kids, which is consistent with the findings of numerous other studies. According to one study, the disease group's nasal *Corynebacteriaceae* were reduced (Cardenas PA *et al.*, 2012). *Staphylococcus, Alloiocococcus,* and *Corynebacterium* had inverse relationships with (lower) RTIs (Teo *et al.*, 2018) .

On the other hand, a shift in the microbiota from *Dolosigranulum* and *Corynebacterium*-dominated profiles to *Moraxella*-dominated profiles at the onset of symptom progression was linked to a higher chance of experiencing a subsequent asthma exacerbation (Zhou *et al.*, 2019). This further suggests that *Corynebacterium* and *Dolosigranulum* play a protective role in the progression of symptoms and subsequent exacerbations. However, numerous microbiota, including

S. saprophyticus and *S. epidermidis* (CoNS coagulase negative staphylococcus), had a higher abundance in control than in patients.

The CONS classification classifies a diverse group of *staphylococcal* species as being less pathogenic or even nonpathogenic. CoNS are unable to produce the virulence factor "coagulase," which is associated with *S.*

aureus, but instead have a variety of virulence factors that vary depending on the species and strain, allowing many of them to act as well-known opportunistic pathogens In (Becker *et al.,* 2020 and Michels *et al*., 2021). Commensal bacteria of human skin and mucosae, including *S. epidermidis,* are highly prevalent (Hotterbeekx *et al*., 2016) and (Gomes *et al.*, 2014).

As a typical component of the skin's flora, *S. epidermidis* typically has a benign interaction with its host. However, if they get past the host body's defense mechanisms, they can infect the same person with serious diseases. It has been demonstrated that commensals develop specific virulence traits during their pathogenic life cycle (Brown m m. *et al.*, 2020). In 97.1% of the cases, *S. epidermidis* was identified as a component of the nasal core microbiota (Kaspar *et al.,* 2016). *S. lentus* was found in small numbers in the patients, which is consistent with our findings. In contrast, no reports of *S. lentus* isolated from the sinonasal cavities have been found in either asymptomatic or sinusitis patients (Stepanović *et al.*, 2006) .

The rationale behind this variability, as presented by Valverde-Molina and colleagues, Health is largely dependent on the process of microbial colonization throughout the first three years of life, with the first hundred days of life being particularly important. Early microbial dysbiosis is linked to a number of variables, including artificial breastfeeding, cesarean birth, and antibiotic medication. Reduced Phylum Firmicutes abundance in the gut microbiome may be linked to a higher risk of asthma. Recurrent viral infections and the onset of asthma are linked to upper airway microbial dysbiosis, particularly early colonization by *Moraxella spp*. Furthermore, chemicals produced by respiratory system bacteria have the potential to alter the onset and course of asthma (Valverde-Molina *et al.,* 2023).

4.6 association between microbiota & study parameters:

4.6.1.association bacteria species with IL-37 and IL-38 serum level in patient group:

test the correlation between bacterial growth from one side and each of IL37and IL38 from another side , revealed Mean for dependent parameter (IL-37) higher in all bacterial species growth on the contrary of Mean of IL-38 in bacterial species growth was lesser .these trouth revealed a positive significant correlation between IL37and all bacterial species growth where (p. value = 0.005). From another point of view, also found relationship in IL-38 and bacterial growth with significant differences (pvalue - 0.006).

In contrast to our findings, a study by (Sokolova *et al.,* 2019) revealed a significant decrease in IL-37 activity in AR (allergic rhinitis). In past study revealed The activity of another member of the IL-1 family, IL-18, in the skin, which is activated through TLR signaling in response to Staphylococcus aureus infection—a pathogen that is frequently present in large quantities on the skin of AtD patients—may be the cause of the IL-37 that was induced both locally and systemically in patients with AtD (Proshkina *et al.,* 2017).

Specifically, IL-37 concentrations in AR patients' nasal lavage and systemic circulation were shown to have significantly decreased (Sokolova *et al.,* 2019). In contrast to our research, we discovered that children with managed BA had significantly lower levels of IL-37 expression in both serum and sputum at both the mRNA and protein levels when compared to healthy volunteers.

Justification Numerous diseases can involve an interaction between the innate immune system and the microbiome. Because microbial products

can persistently trigger innate immune responses and cause chronic inflammation, the microbiota is crucial for tissue health, and changes to it can result in autoimmune and chronic illnesses (Thaiss *et al.*, 2016). Thus, autoimmune illness may result from persistent self-inflammation brought on by changes in the host-microbiota and the innate immunity axis.

Higher blood IL-38 levels in bacterial pneumonia patients are linked to anti-inflammatory responses in respiratory infections, according to a study. This suggests that IL-38 plays a crucial role in reducing excessive lung inflammation in response to exogenous pathogens. What's more, IL-38 showed signs of being a novel potential biomarker for bacterial pneumonia (Sun *et al.*, 2023).

The development of the immune system, lung function, and gut and airway microbiome occur simultaneously, and dysbiosis of the microbiome may have a significant role in the emergence of asthma (Pijnenburg *et al.*, 2022). The pathophysiology of asthma is significantly influenced by the airway microbiome. Variations in the pulmotypes of the asthma-associated airway microbiota are linked to and may impact asthma, namely inflammatory phenotypes (Wang *et al.*, 2022) .

4.6.2.Correlations Bacteria Species with IL-37 and IL-38 Serum level in Control Groups.

In a similar way, table 3.7 evaluates the relationship between bacterial growth on one side as an independent variable and IL37 and IL38 on the other as dependent factors. A cultivation investigation based on the results of the VITEK compact system and morphological analysis revealed that the control group consisted of *C. dephtheria, H. influenza , K.rosia, S. aureus, S. epidermidis, S. mutans, S. pyogen, and S. saprophyticus*. This revealed that the mean for the dependent parameter (IL-37) was higher in all bacterial species growth, while the mean for IL-38 in bacterial species

growth was lower. The biomarker and bacterial growth in the control group did not differ significantly.

Under normal conditions, some bacterial species, such as *S. aureus*, have been suggested to play a protective role in the sinus microbiome; however, when dysbiosis is present, their presence is associated with both a strong local immune response and the severity of the disease (Schwartz *et al.*, 2016).

The principal natural reservoir of *S. aureus* is human asymptomatic carriage, and the major ecological niche in over 50% of the general population is assumed to be the anterior nasal mucosa and skin (Diep *et al.*, 2006).

Early in infancy, the host immune system's development can be significantly influenced by the gut microbiota (GM) (Deretic and Levine, 2009).Firmicutes, Actinobacteria, and Bacteroidetes are the three phyla of bacteria that make up the healthy lung microbiome.(Hufnagl *et al.*, 2020). It is becoming more and more clear how important it is for children to be exposed to their surroundings during a key window of time when immune maturation and microbiome development take place (Pijnenburg & Associates, 2022).

Patients with bronchial asthma had significantly greater serum IL-38 levels than the control group ($p < 0.001$) (Kamal and others, 2022). IL-37 production by stimulated PBMCs taken from children with aBA was significantly lower than that of healthy volunteers, according to research on IL-37 in aBA (Baird *et al.*, 2018).

When compared to healthy volunteers, there was a substantial drop in IL-37 expression in the blood, sputum, and mRNA and protein levels of 40 children with managed BA, almost 70% of whom had allergic asthma (Shilovskiy *et al.*, 2019). Firmicutes, Actinobacteria, and Bacteroidetes are

the three phyla of bacteria that make up the healthy lung microbiome (Hufnagl *et al.,* 2020).

According to a study on childhood asthma, children's immune system development is significantly impacted by early exposure to pathogens. The original "hygiene hypothesis" has been further supported and improved in light of changes in modern lifestyles, such as an increase in the number of cesarean sections performed, an increase in the use of antibiotics, the widespread use of formula feeding, and changes in modern dietary patterns, all of which have a significant impact on the components of the gut microbiome (Frati *et al.,* 2018) and (Daley, 2014).

4.7. bacterial types among asthma severity class in patient group .

Bacterial types after diagnostic according to VITEK2 compact system compare with relationship to asthma severity(mild,modrate,severe) shown in table (3.10).

In patients suffering from severe asthma and similar phenotypes, some bacteria are linked to and have the potential to regulate inflammatory processes. When inhaled corticosteroid treatment is combined with severe asthma, patients' airway dysbiosis seems to be different from that of individuals with milder asthma . Major human pathogen *Streptococcus pyogenes (S. pyogenes)* produces a variety of infections, ranging from mild to severe and invasive diseases with significant morbidity and mortality (Iqbal *et al.,* 2023).

our study found that *Pseudomonas* was more prevalent in moderate asthmatics compared to those with mild and severe asthma (p-value 0.39). In contrast to earlier studies that found that *Pseudomonadaceae* and *Enterobacteriaceae* were more prevalent in severe asthmatics compared to non-severe asthmatics ($p < 0.05$), (Li *et al.,* 2017) .

However, our research supported previous findings showing, when assessed in samples obtained by bronchscopy, the airways of people with moderately severe asthma have higher levels of Proteobacteria than those of normal controls (Hilty *et al.*, 2010).

Proteobacteria, primarily *Haemophilus*, but also occasionally *Klebsiella*, *Neisseiria*, and *Moraxella* species, are identified in mild to moderately severe asthma cases (Hilty *et al.*, 2010), (Marri *et al.*, 2013) and (Huang *et al* ., 2015). These results are consistent with our findings that *Moraxella* and *Haemophilus* are associated with mild to moderately severe asthma**.**

However, a different investigation yielded results that contradicted our findings. *Haemophilus* influenzae has been found in patient samples with severe cases that are inadequately managed (Chung, 2017 and Simpson *et al.*, 2016).

Compared to controls, severe asthmatics had higher incidence of firmicutes, especially from an increase in *streptococci* (Zhang *et al.*, 2016).These results are in line with those in our report, which indicated that *streptococcus*, particularly *s. pneumonia*, was more closely associated with asthma severity in individuals with moderate to severe asthma (p-value 0.021**).** Additionally, our results are in line with a study's findings, which showed that asthmatics' enrichment in the genus *Streptococcus* and the phyla *Proteobacteria* and *Firmicutes* likely influences the onset and severity of asthma (Losol *et al.*, 2021**).**

According to (Davis *et al.*, 2015), there is a correlation between an increase in the severity of asthma and *S. aureus* colonization of the nasal cavity. In line with our findings, it was discovered that children with moderate-to-severe asthma had higher levels of germs.

The justification behind these Through its proteins, *S. aureus* modifies the immune system of the airway mucosa on multiple levels. It does this by releasing IL-33 from the respiratory epithelium and inciting the activation

of innate lymphoid cells (ILC), which in turn triggers the release of type 2 cytokines by those ILC and T helper (Th) 2 cells, degranulation of mastocytes, the massive activation of local B cells and the formation of IgE, and, lastly, the attraction of eosinophils and the ensuing liberation of extracellular traps. All of these mechanisms exacerbate epithelial damage and increase the persistence, exacerbations, and severity of the disease (Krysko *et al.*, 2019), (Bachert *et al.*, 2020), and (Stentzel *et al.*, 2017).

A prior investigation revealed It was discovered that there were differences in the spectrum of composition of the nasopharyngeal microbiome between the asthmatic group and the control group (p=0.007), as well as between individuals with varying degrees of asthma severity for *K. rosea* (p= 0.02). The results validate previous reports (Teo S. M. *et al.*, 2015 and M.L. von Linstow *et al.*, 2013) that showed differences in the microbiome makeup between people with aggravated asthma, those without exacerbated asthma, and healthy controls.

It was discovered in one study that those with more severe asthma had a stronger correlation (p=0.02). Gram-positive, catalase-positive, and oxidatively positive cocci are known as *K. rosea*, Malachite green and other commercial colors are reported to be biodegradable by K. rosea (G. K. Parshetti *et al.*, 2012 and G. K. Parshetti *et al.*, 2010). These two studies on K. rosea were relevant to ours, as we found that children with severe asthma had a high abundance of bacteria (p-value <0.001).

The explanation for the correlation between the severity of asthma and bacteria has been demonstrated by numerous studies. For example, the bacteria in the respiratory system create metabolites that may alter the onset and course of asthma (Valverde-Molina *et al.*, 2023). According to(Zhou *et al.*,2019), (Zhang *et al.*, 2016), and(Barcik *et al.*, 2020), there is a correlation between airway microbial dysbiosis and the development, exacerbations, and response to treatment of the disease,

Dysbiotic communities can significantly influence the course and severity of asthma.

Based on all of these research, it appears that the microbiota plays a major role in regulating immunological, metabolic, and cell processes. It reacts to inflammatory signals linked to asthma and likely plays a mediating role in the severity, phenotype, and susceptibility to asthma (Ver Heul *et al.*, 2019). Since the foundation of therapy is inhaled steroids, the cause of asthma that does not respond to treatment may be explained by the involvement of specific bacterial genera in the mechanisms of resistance (Valverde-Molina *et al.*, 2023) .

4.8. Correlations Between Bacterial Species and Demographic Characteristics.

The current investigation's findings indicate that both genders experience the same prevalence of bacterial growth (female 41.6% and male 58.4%). Some bacteria showed significant differences according to gender within the study groups. Significant differences exist among *S. aureus, S. mutans,* and *S. pneumonia* (p-values of 0.04, 0.02, and 0.02 in that order). in order for the differences in gender seen in any other bacterial growth across the study groups to not be statistically significant.

One study's findings supported the current investigation. One of the most common chronic lung diseases, asthma usually starts in early childhood. An increased risk of developing asthma in later childhood is linked to the colonization of newborn airways by the pathogenic bacterial strains *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pneumonia* (Larsen *et al.*, 2014).

From a broader viewpoint, sex-related variations in the prevalence and severity of asthma can also be taken into account. Males and females have different susceptibilities to numerous diseases, as recent research has

demonstrated. Female immune systems generally react to common bacterial and viral infections more effectively (Jacobsen *et al.,* 2021 and Dias *et al.,* 2022).

In contrast to men, females may be more vulnerable to more severe consequences from inflammatory lung disorders due to the differential effects of sex hormones on lung immune responses, airway responsiveness, and pulmonary circulation (Mokra *etal.,* 2023).

The explanation is that while testosterone has the opposite effects, estrogen stimulates the production of pulmonary surfactant and the transition from the terminal saccular stage to the alveolar stage, reducing the susceptibility of female neonates to infection and speeding up lung maturation (Gortner *et al.,* 2013) .

However, the investigation's findings on bacterial growth showed that, with respect to age, all age groups exhibited bacterial growth with *S. pneumonia and S. saprophyticus* at age levels of 1-4 years in more significant differences (p-values of 0.01 and 0.01, respectively). study demonstrated the intimate relationship between these germs and the individual at that age (stronger in the case of the 4-year-old). Additionally, various bacterial growths, such as *C.deptheria , H.influenza , K. rosia, M.catarrhales, P.earoginosa, S. aureus, S. epidermidis, S. lentus, S. mutans, S. pyogen, and S. xylose,* occurred at that age with non-significant differences.The p-values are 0.29, 0.93, 0.30, 0.17, 0.69, 0.43, 0.70, 0.74, 0.36, 0.53, 0.74.

Numerous studies have been found to support the current investigation. Three recent, unbiased analyses of preschool-aged children (less than five years old) with recurrent wheezing have revealed distinct clusters of inflammation in the lower airways, with a predominance of eosinophila linked to aeroallergen sensitization or neutrophilia linked to bacterial and/or viral infection(Robinson *et al.,* 2021).

Reduced diversity and a high abundance of *Moraxella* in the microbiota have been linked to asthma and preschool wheeze; on the other hand, greater bacterial diversity has been positively correlated with protection (Birzele *et al,.* 2017) and (Depner *et al.,* 2017).

The development of asthma ten years later has been linked to the makeup of indoor dust and bacterial exposure at two months of age (Karvonen *et al.*, 2019). All bacterial collections develop most aggressively in the winter and fall and less so in the summer, according to the investigation's findings. *S. aureus and S. pneumonia* had higher growth in the fall and winter but lower growth in the summer when compared to other normal flora in these seasons (p-values 0.06 and 0.05, respectively). Comparably, *S. saprophyticus* showed strong growth in the winter but less in the autumn and no growth in the summer (p-value 0.03), and *S. mutans* showed strong growth in the winter but no growth in the summer or autumn with significant differences (p-value 0.02). Table 3.10 shows that these normal flora differ significantly with respect to different seasons.

These studies corroborated the findings that the composition of the nasal microbiota varies with the seasons and that the likelihood of an asthma attack is influenced by specific microbe–host interactions. Variations in the baseline and respiratory disease microbiota were found to be associated with a subsequent exacerbation in a seasonally adjusted study. Particularly, a number of *Moraxella* and *Haemophilus* members were more prevalent in respiratory infections that were virus-positive and those that developed into exacerbations throughout the fall, when these occurrences were most common (McCauley *et al.,* 2022).

4.9. Typing Of Bacterial Sample According To Gram Stain.

Gram staining, either with or without residual gram dye, was employed in this experiment to classify bacterial samples into two groups: gram positive

(purple-colored) and gram negative (red-colored). Table 11 displays the gram positive and negative distribution between patients and controls. In all research groups, the percentage of gram negative individuals is lower than the percentage of gram positive individuals (17.0% gram negative, 83.0% gram positive). further observing noteworthy variations (p-value 0.042) between the two groups.

According to this study, the percentage of microorganisms by Gram's staining in cases of chronic bronchitis, asthma, and controls was 86.67%, 76.67%, and 93.33%, respectively. In contrast, only 76.67% of the control cases had bacteria. In the majority of instances, the morphology of the bacteria detected by gram staining was also verified by culture (Solanki *et al.* 2018). Contrary to research by (Ege *et al.,* 2012) discovered a negative correlation between the onset of asthma and both gram-positive (*staphylococci, corynebacteria*, lactic acid fermenters) and gram-negative (*neisseriae, Acinetobacter*) bacteria while comparing dust from rural and suburban locations in Germany .

4.10 Distribution Studying Group In Relation To Bacterial Type .

In the current investigation, the association between the study group and the bacterial typing (gram positive, gram negative) is assessed after the bacterial sample was typed using gram stain into gram positive and gram negative categories. When gram positive bacteria were more prevalent in patients than gram negative bacteria, with trends for *S. aureus, S. pneumoniae, and K. roseia* (31.6%, 28.9%, and 13.2%, respectively). However, among gram-negative bacteria, *H.influenza* and *M.catarrhales* have been trending at 50.0% and 41.7%, respectively. these groups had a p-value of 0.002**, which is extremely significant. However, gram positive bacteria are also more prevalent in control groups than gram negative ones, with *S. Pyogen, S. Epidermidis, and S. Saprophyticus* rising higher in this

group (22.2%, 20.0%, and 28.9%, respectively). *H. influenza*, however, was the only gram-negative bacterium (100.0%) in the control group. Thus, there are very significant differences between these groups (p-value 0.001**).

Likeness to the ongoing research that we discovered Of the 273 patients, 12.1% had positive bacterial cultures. A variety of bacterial species were present in the flora, including *Neisseria* species (7.9%, 3/38), *S. pneumoniae* (7.9%, 3/38), *M. catarrhalis* (23.7%, 9/38), P. non-aeruginosa (5.3%, 2/38), and *P. aeruginosa* (2.6%, 1/38), It is noteworthy that more than 90% of the *B. catarrhalis* isolated in this investigation and 46.7% of the *H. influenzae* generated beta lactamase. The latter, formerly known as *M.catarrhales*, is a gram-negative aerobic bacterium that was once believed to be a harmless commensal of the human upper respiratory system. It is worth more attention (Jacobsen *et al.,* 2021) . Of the patients, 70.9% had isolates, and 24 (43.6%) had *S. aureus* that was resistant to antibiotics (MRSA). Major species detected were *S. aureus* (55.6%) and *S. epidermidis* (26.5%) (Augusto de Oliveira *et al.,* 2024).

4.11 distribution bacterial swabs between studying group in relation to number of isolate .

4.11.1 single or multiple bacterial isolate of patient group.

By using a laboratory method to collect samples for the patient group, we found that swabs with double bacterial type had the highest number (28) of all culturing swabs with percentage (56%), followed by swabs with single bacterial type (20) with percentage (40%), and finally, swabs with triple bacterial type (2) with percentage (4%) which may be from the same species or different. As a result, no swabs had quadrate bacterial sample shown during sampling collection.

When compared to the healthy groups, the upper respiratory tract samples from asthmatics typically exhibited reduced alpha diversity (Birzele *et al.,* 2017and Majak *et al.,* 2021). According to Thorsen , nasopharyngeal relative abundance values of *Streptococcus pneumoniae*, *Haemophilus influenzae, and Moraxella catarrhalis* are connected with the same species in the hypopharyngeal cultures. These findings show that nasopharyngal swabs have several bacteria that are identical to the current invasion(Thorsen *et al.,* 2023). Additionally, in a study that resembles the current investigation by using bacteria from nasopharyngeal swabs in patient groups, colonization by *Moraxella catarrhalis* was particularly high (92.1% of children on ≥1 swab), followed by Haemophilus influenzae (87.1%) and *S. pneumoniae* (83.2%) (Pol *et al.,* 2024). Using nasopharyngeal swabs as opposed to oropharyngeal swabs resulted in considerably greater isolation rates of *S. pneumoniae* and *M.catarrhales* species (Odutola *et al.,* 2013).

that many studies were published Children who have a higher chance of experiencing recurrent wheezing and developing asthma in the future were found to have nasal samples with high concentrations of *Moraxella* collected from wheezers either during enrollment or during respiratory infection (Dumas *et al.,* 2019), (Mansbach *et al.,* 2020), and (Tang *et al.,* 2021). Remarkably, clusters of the *Corynebacterium* genus in the nasal samples were linked in certain studies (Zhou, *et al.,* 2019) and (Hou *et al.,* 2022) to a reduced asthma exacerbation/disease-control loss ratio.

The explanation for this variation in study groups is related to the presence of positive bacteria in the upper respiratory tract, which are beneficial to the individual and have a normal microbiota. However, they can also become pathogenic when taken in excess or alter the epithelial environment due to repeated RSV infections, colonization by numerous opportunistic bacteria, or allergies.

4.11.2 Single Or Multiple Bacterial Isolate Of Control Group.

Through the laboratory method used to gather the samples for the control group, we found that the largest number of swabs (29), with a percentage of 58%, came from culturing single bacterial type swabs. Next, swabs with double bacterial type (which could be from the same genus or different), had number (18) and a percentage of 36%. Finally, swabs with triple bacterial type (which could be from the same species or different) had only view number (3) with percentage (6 %), meaning that no swabs had quadrate bacterial sample showed during sampling collection.

the Center for Disease Control and Prevention (CDC) recommends "gently rubbing and rolling" the swab after reaching the nasopharynx and leaving it in place for several seconds before withdrawing it (Center for Disease Control Prevention, 2022).

In such study, the relative abundance of bacteria was significantly higher in asymptomatic and mild patients compared to severe patients and the control group. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteriales* were the most abundant phyla in the positive groups, while Actinobacteria, *Proteobacteria*, and *Firmicutes* were dominant in the control group (Hyblova *et al.*, 2023).

Conclusion

&

Recommendations

CONCLUSION

1. There is a marked elevation of levels of both IL37 & IL38 in recurrent wheezers which may serve as inflammatory markers or a precursor to the disease process. Both IL37 & IL38 could be considered good diagnostic biomarkers for recurrent wheezers.
2. The combination of Staph. aureus and Strep. pneumoniae was higher in asthmatic patients, while coagulase-negative staphylococci (CONS) were considerably higher in healthy controls. Gram-positive bacteria were abundant in Both study groups. 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.
3. There is a significant association between the type of bacterial microbiome and serum levels of IL37 & IL38 among the patient group. This may suggest the role of dysbiosis in development of asthma and dysregulated immune response.
4. Haemophilus species were associated with mild asthma, k. rosia were more in severe asthma while s. pneumonia is associated with moderate asthma.

Recommendations

- Follow-up studies are recommended, inorder to track the effect of dysbiosis on children with asthma.
- Follow-up studies for possible changes in microbiota with age and severity of asthma are recommended.
- Studying the effects of the microbiome on more immunological markers is recommended.
- Studies correlating IL37 & IL 38 with asthma phenotypes are recommended.

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Appendix

Appendix 1: Questionnaire

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

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

. . . ..……...Age : . . . . . .

male ☐ female ☐ Address : . . . . . .

Weight : . . . . . kg Height:. . . . . .cm

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	
Drug allergy :	Yes	☐	NO	☐
Type :				
Food allergy :	Yes	☐	NO	☐
Type :				

Attacks/year: 3 & more ☐

< 3 ☐

Response to bronchodilator: good ☐ bad ☐

Nebulizer use: yes ☐ NO ☐

Covid19 yes ☐ No ☐

family h. Of asthma yes ☐ No ☐

Aggravating factors :

Viral infection yes ☐ no ☐

dust exposure yes ☐ no ☐

cold air yes ☐ no ☐

fume yes ☐ no ☐

Exercise yes ☐ no ☐

Playing yes ☐ no ☐

Passive smoking yes ☐ no ☐

Animal in the house: Cat…….. dog ……chicken…….Birds…….Cows…… sheep……..

Treatment:

Montelucast ……☐……. ICS……☐……

Level of control:

Well ☐ not well ☐ very poorly ☐

Lab. Parameters

eosinophil count……. Neutrophil count……. Lymphocyte count……

Lab .investigation :

Swab microbiological VITEK2 result……

IL-37……

IL-38……

Total IgE……..

Appendix 2

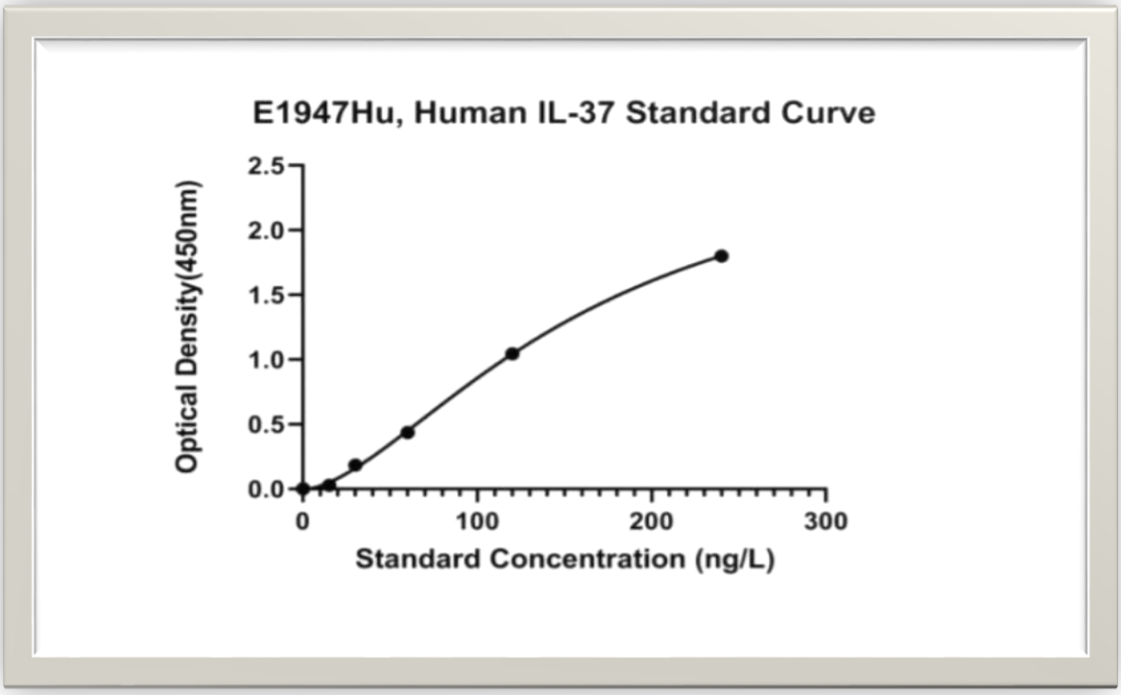


Figure (1): The standard curve of IL-37.

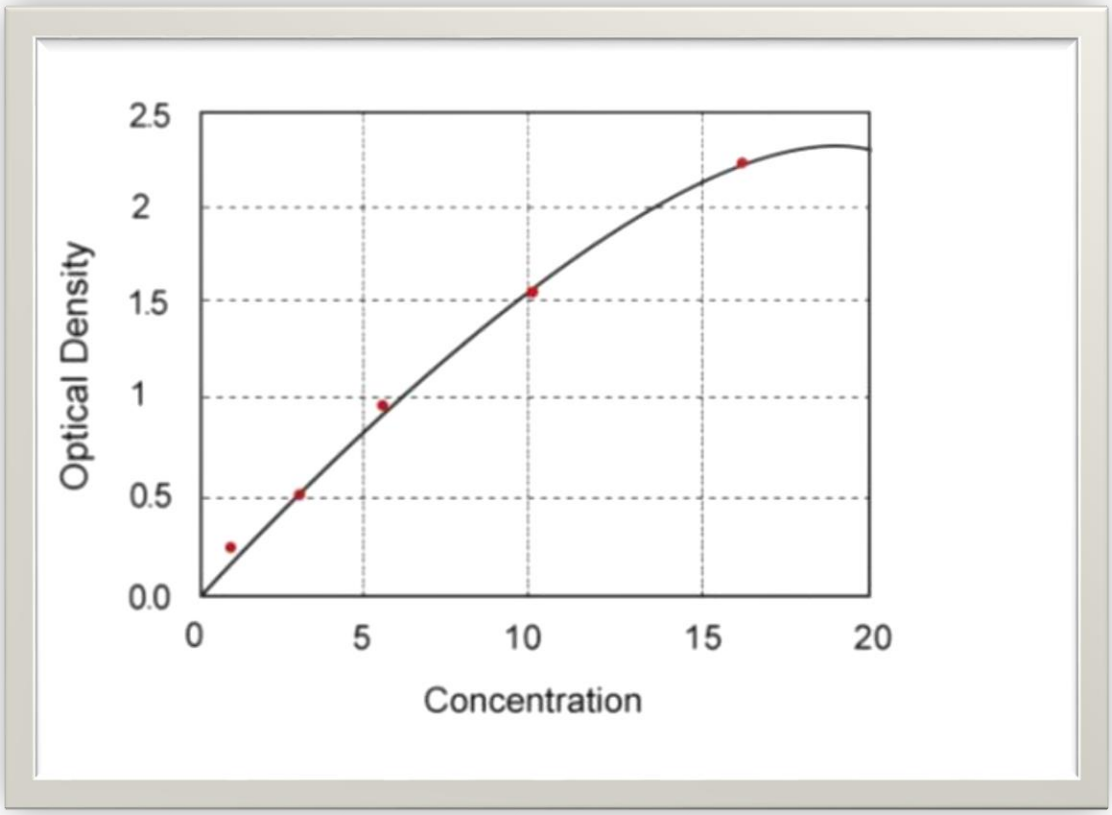
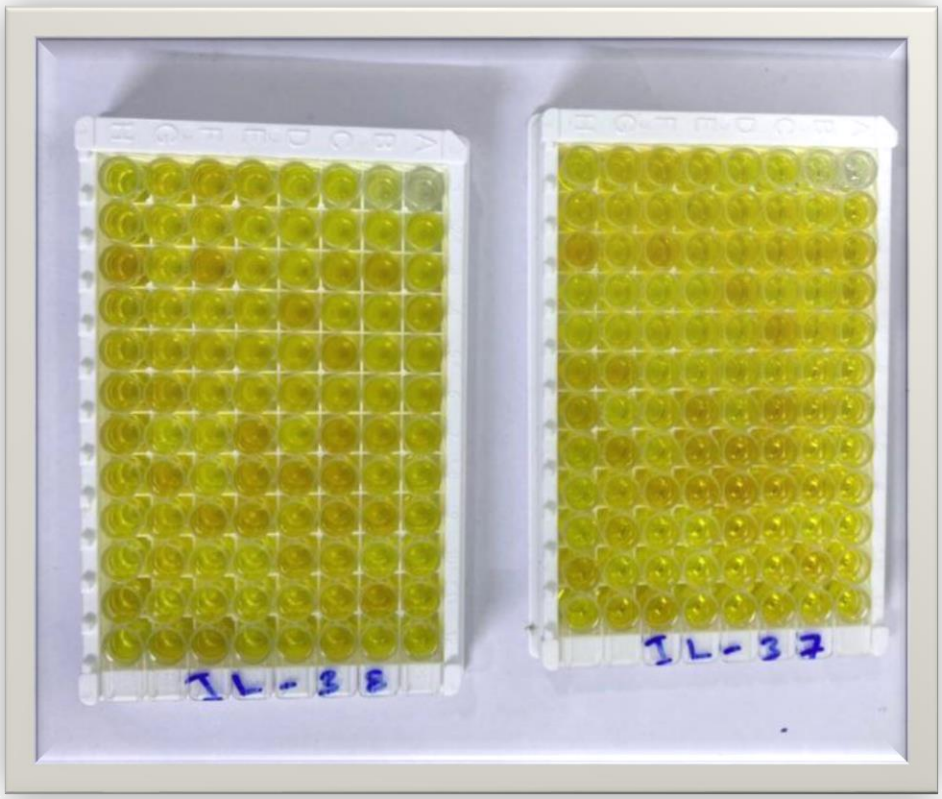
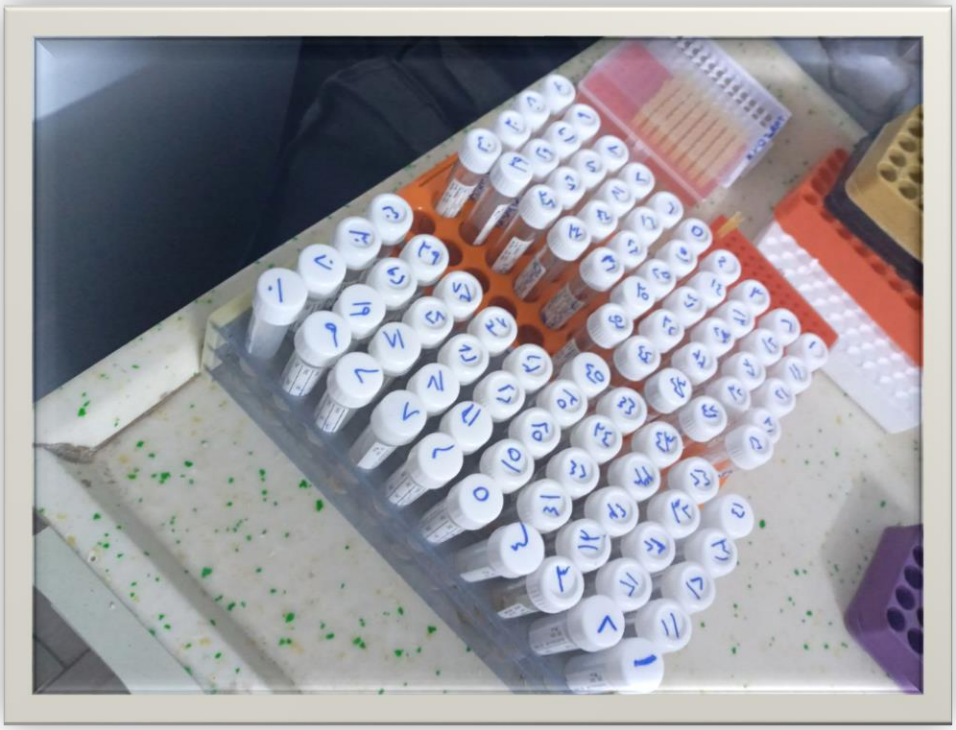


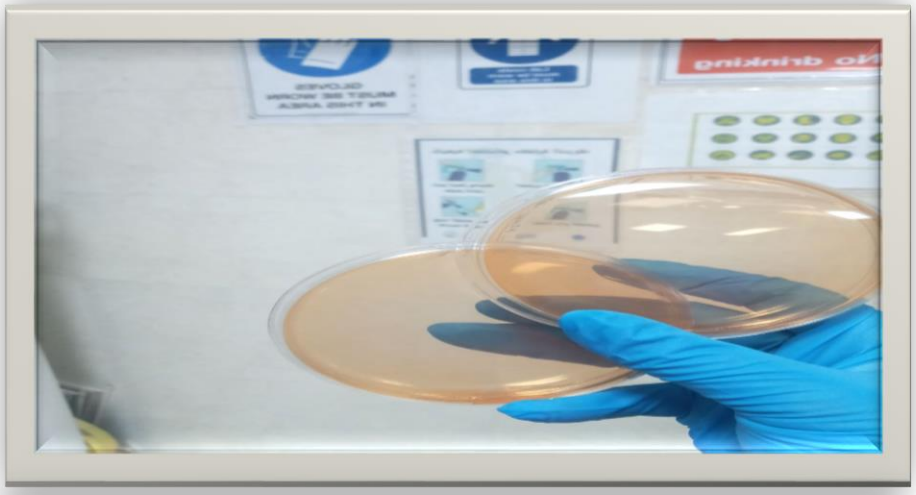
Figure (2): The standard curve of IL-38

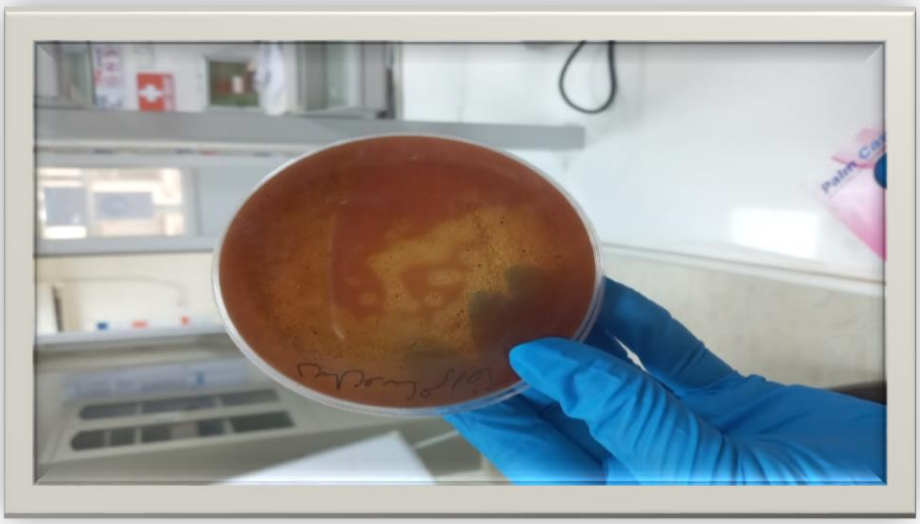
Appendix (3)



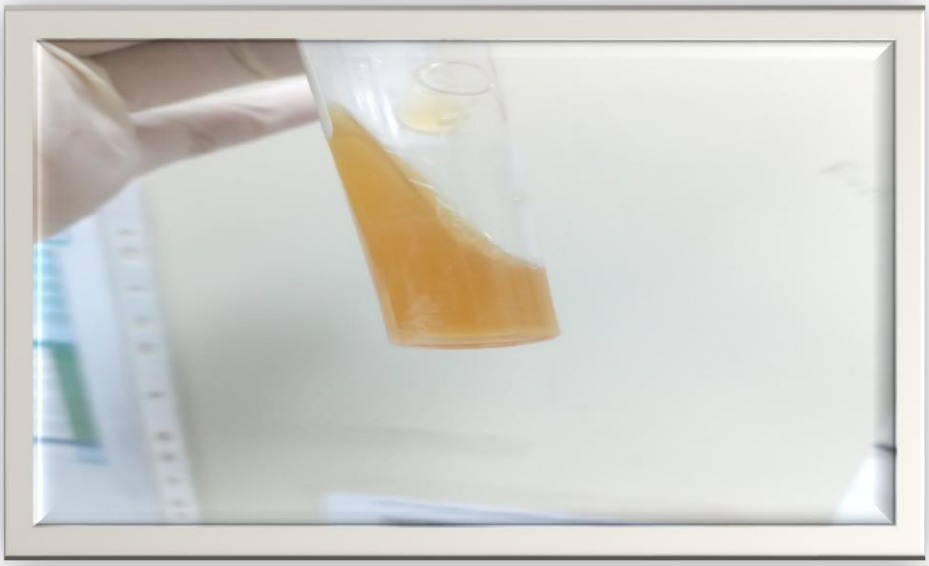


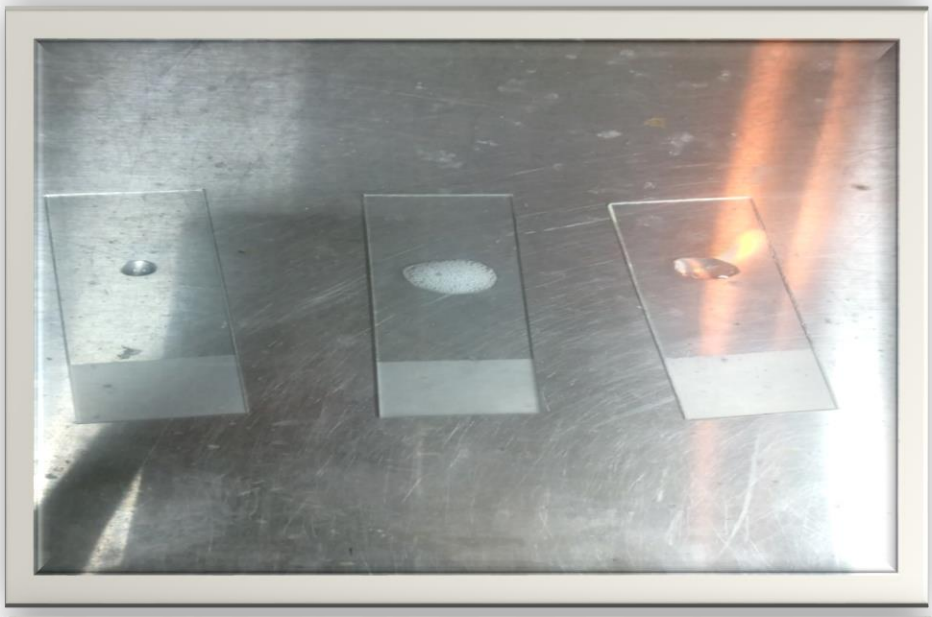
Appendix (4)



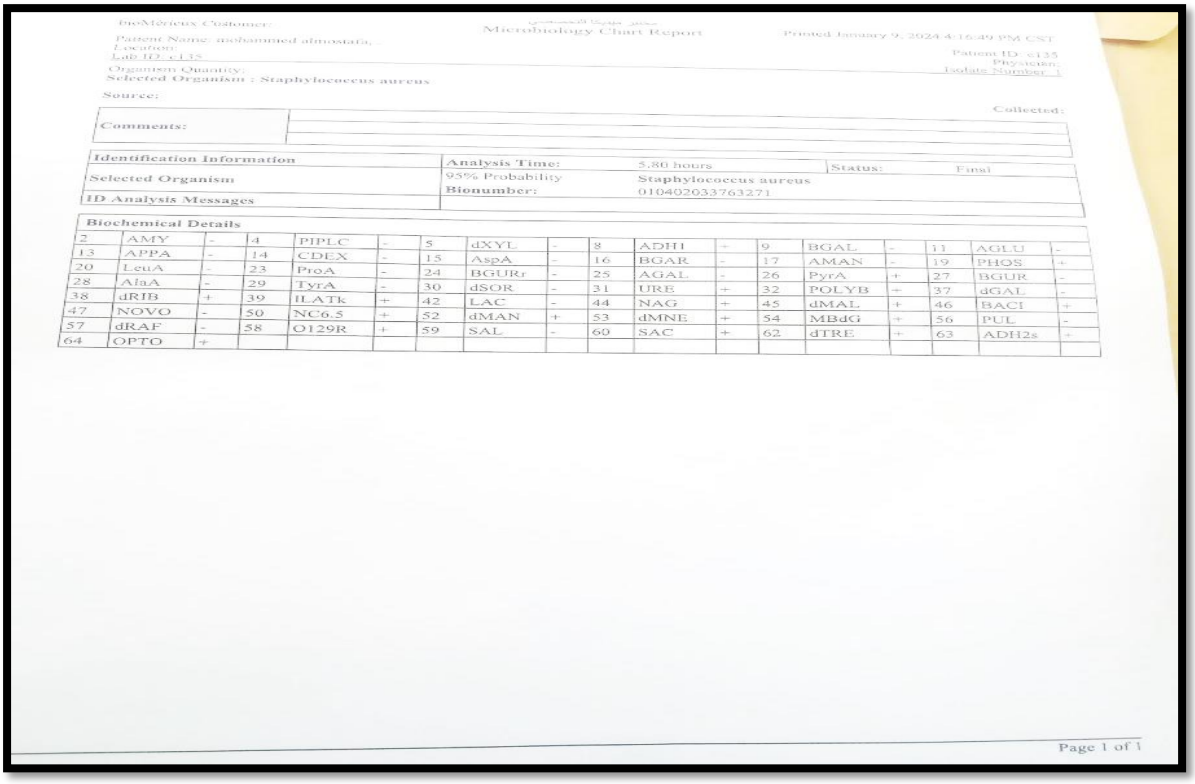


Appendix (5)





Appendix (6)



bioMérieux Customer:

Microbiology Chart Report

Printed January 9, 2024 4:15:49 PM CST

Patient Name: mohammed almostafa,
Location:
Lab ID: c135

Patient ID: c135
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source:

Collected:

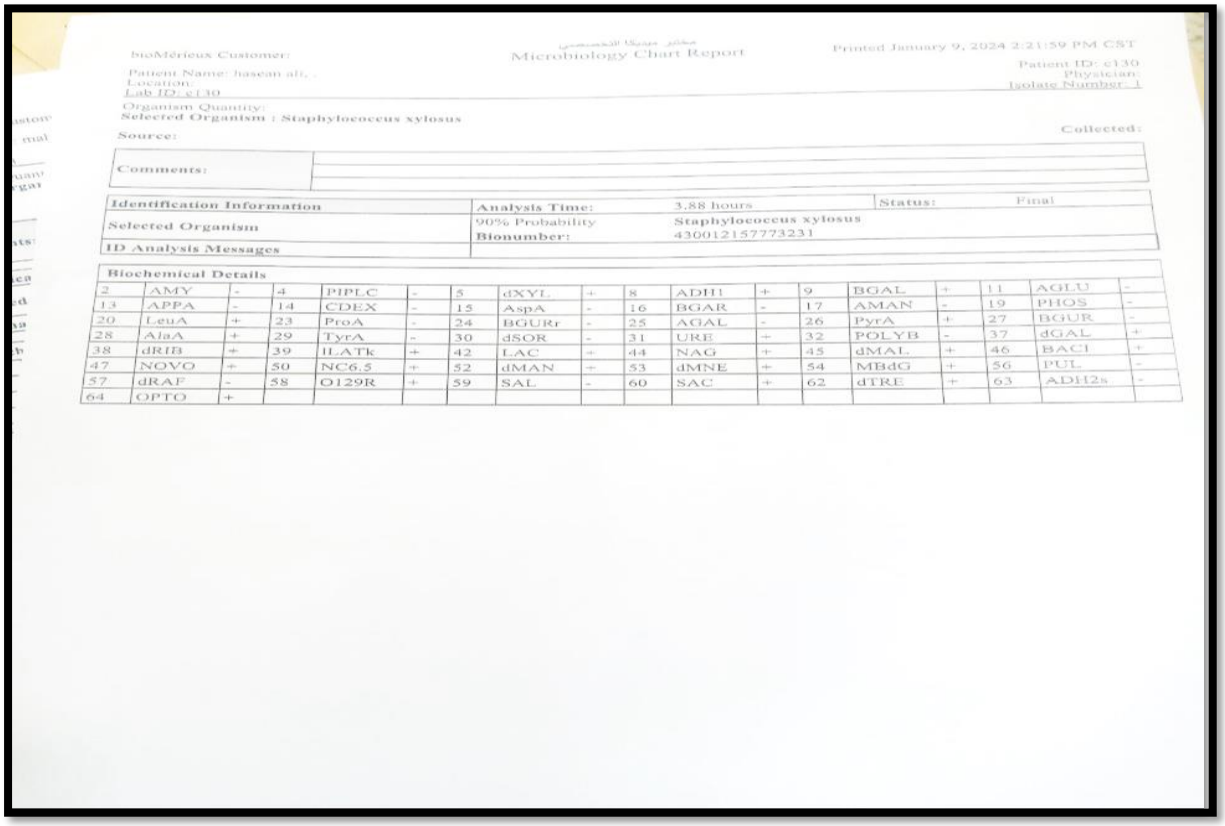
Comments:	

Identification Information	Analysis Time:	5.80 hours	Status:	Final
Selected Organism	95% Probability	Staphylococcus aureus		
ID Analysis Messages	Bionumber:	010402033763271		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	-
38	dRIB	+	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Page 1 of 1



bioMérieux Customer:

Microbiology Chart Report

Printed January 9, 2024 2:21:59 PM CST

Patient Name: hasean ali, .
Location:
Lab ID: c130

Patient ID: c130
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus xylosus

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	3.88 hours	Status:	Final
Selected Organism	90% Probability	Staphylococcus xylosus		
ID Analysis Messages	Bionumber:	430012157773231		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix 7


BT LAB
Bioassay Technology Laboratory
Human Interleukin 37,IL-37 ELISA Kit
2-8°C
Size: 96T
Cat.No.E1947Hu
2024/1/4
2025/1/3
202401003
FOR RESEARCH USE ONLY
BT LAB
Bioassay Technology Laboratory
Human Interleukin 38,IL-38 ELISA Kit

الخلاصة

وفقًا لأحدث إصدار من إرشادات المبادرة العالمية للربو، فإن الربو هو "مرض غير متجانس، يتميز عادةً بالتهاب مجرى الهواء المزمن. يُستخدم مصطلح الصفير المتكرر الآن بشكل شائع للأطفال الذين تقل أعمارهم عن 3-5 سنوات. العلاقة بين البكتيريا الطبيعية والربو معقدة، وتشير الأدلة إلى أن بعض الكائنات الحية الدقيقة المرافقة قد تلعب دورًا في تطور الربو. قد يلعب التعرض لبعض الميكروبات البيئية أثناء الطفولة المبكرة دورًا حاسمًا في تعزيز نمو الجهاز المناعي الطبيعي ومنع الربو. تتأثر العمليات المؤدية إلى تطور الربو بشكل كبير بالتفاعل بين الجهاز المناعي للمضيف والميكروبات

أجريت دراسة مقارنة لمدة 6 أشهر، ابتداءً من أغسطس/آب 2023 إلى يناير/كانون الثاني 2024، بلغ إجمالي عدد المشاركين 100 مشارك، تم تقسيمهم إلى مجموعتين: الأولى تضم مرضى الربو عند الأطفال و/أو الصفير المتكرر، بينما تضم المجموعة الثانية الاطفال الاصحاء. تم إجراء الفحوصات المخبرية بتقنيات مصلية (اختبار الإليزا الساندويتش)، وتم فحص العينات لمعرفة مستويات IL-37 وIL-38 وIgE في المصل البشري. تم أخذ مسحات من جميع المشاركين وإجراء فحص بكتيري (زراعة مورفولوجية ومجهرية واختبار تأكيدي بنظام VITEK 2 compact).

وقد ظهرت فروق معنوية في متوسط مستويات IL-37 و IL-38 في المصل ضمن المجموعات المدروسة، حيث ارتفع كلا المؤشرين بشكل ملحوظ في مجموعة المرضى، بالإضافة إلى أن جميع معايير الدم (عدد الخلايا الليمفاوية، الخلايا الحمضية، الخلايا المتعادلة) لها مستويات معنوية عالية ضمن مجموعات المرضى. وقد أظهر تحليل منحنى ROC أن IL-37 و IL-38 قادران على التمييز وتشخيص المرضى الذين يعانون من الصفير المتكرر من الاشخاص الصحاء. وبالتالي يمكن اعتبارهما مؤشرين حيويين مفيدين للتشخيص.

أظهرت نتائج الفحص الزراعي المعتمدة على النتائج المورفولوجية ونتائج نظام VITEK compact أن مجموعة المرضى أظهرت نموًا بكتيريًا على النحو التالي : Staphyloococcus. aureus بنسبة % (24.00) 12 ثم Streptococcus. Pneumonia 11(22.00) تليها % H. influenza 6 (12.00) و % K. rosia 6 (12.00) ،

Staphylococcus. epidermidis ، Moraxella.catrrales 5 (10.00) % Streptococcus. pyogen 3 (6.00) % ، 4 (8.00) % ، وأخيرًا P.aerogenosa 1 (2.00)% و S. xylose 1 (2.00) %.

في حين سجلت مجموعة الاصحاء أن Staphylococcus .saprophyticus. كانت هي البكتيريا الأكثر عزلة في هذه الدراسة بنسبة % (25.50) 12 تليها Streptococcus. pyogen بنسبة % (19.60) 10 ثم Staphylococcus. epidermidis بنسبة% (19.60) 10 و Streptococcus. mutans بنسبة % (13.70) 7و Haemophilus.influenza بنسبة % (9.80) 5 و Staphylococcus. aureus بنسبة % (5.90) 3 وأخيراً Kocuria. rosea بنسبة % (3.90) 2 و Corynebacterium.dephtheria بنسبة % (2.00) 1.

وفي الاستنتاج، هناك ارتفاع ملحوظ في مستويات كل من IL37 وIL38 في حالات الصفير المتكررة والتي قد تعمل كعلامات التهابية أو مقدمة لعملية المرض ويمكن اعتبارها علامات تشخيصية جيدة للصفير المتكرر. كان مزيج Staph. aureus وStrep. pneumoniae أعلى في مرضى الربو، في حين كانت المكورات العنقودية السلبية لانزيم التخثر (CONS) أعلى بكثير في الاصحاء . يمكن أن يعزز خلل التوازن الميكروبي استجابة مناعية غير متوازنة ويساهم في تطور اضطرابات الحساسية، وخاصة الربو.

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وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

فرع الاحياء المجهرية الطبية

تأثيرات الميكروبيوم البكتيري التنفسي على مستوى مصل IL-37 و IL-38 مع الارتباط مع شدة الصفير المتكرر

رسالة مقدمة الى

مجلس كلية الطب وهي جزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية الطبية

من قبل الطالبة

ذوالنورين حسن هادي الجشعمي

بكالوريوس تحليلات مرضية (2019)

بأشراف

الاستاذ الدكتورة	الاستاذ المساعد الدكتورة
سوسن محمد جبار الحسناوي	مي محمد علي

1446 هـ

2024 م