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The Role of MicroRNAs in Respiratory Disorders: Exploring the Connection between IL-13, miR-625, miR-151, and Microbial Infection

A thesis Submitted to the Council of the College of Science for Women at Babylon University as Partial Fulfillment of the Requirements for the Degree of Master in Biology

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بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا

صدق الله العلي العظيم

سورة الاسراء : 85

Dedication

To the glory of Allah, who is both my creator and my master; To Mohammed, my great teacher and messenger (may Allah bless him), who enlightened us on the meaning of life.

To my dear husband, who is the best support for me in my studies.

To my wonderful parents, who have been my source of strength, confidence, and unwavering support....To all of my family, my friends, and everyone else that I love.. To my motherland of Iraq.

I would want to dedicate this study to everyone who assisted me in every way imaginable in order to bring this project to fruition.

Rana

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My deepest and most sincere gratitude goes to the respiratory disorder patients from whom I was able to collect samples; may Allah

Rana

Certification of the Supervision

I certify that this dissertation entitled(Immunogenetic assessment and relationship with microbial infection for respiratory disorder patients) " was prepared by Rana Kadum Kareem Al-maamory, under our supervision at the Department of Biology College of Science for Women, University of Babylon, as a partial fulfillment of the requirements for Degree of Master in Science of Biology

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Data: /. / 2023

Signature

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In the view of the available recommendation, I forward this thesis for debate by the examining committee

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Summary:

Respiratory disorder refers to a wide range of condition that effect the upper and lower respiratory diseases. upper respiratory system includes sinusitis, colds, epiglottis tall and pharyngitis, lower respiratory diseases include Asthma, Coronavirus، tuberculosis (TB), pneumonia. Cause of respiratory diseases is Bacteria, virus Fungi and environmental condition.

The aim of study is determining the concentration of IL-13 by using Elisa Technique, estimation of Some microRNA roles (625, 151 genes) in different type، respiratory disease by using RT QPCR and know kinds of bacteria that effect respiratory system. One hundred fifty-five samples varied between infected and healthy were collected in AL-Mahaweel, Marjan hospital and so AL-Mukhtar center for Tuberculosis Babylon province/Iraq. During the period from September 2022 to February 2023. Samples divided as Follow A. Total of one hundred seven specimen of Samples have been collected from patient with respiratory disorder. Samples distributed into Some -diseases including (Asthma, Corona virus, Bronchitis, Sensitive Bronchitis and Chronic Obstructive pulmonary) Exclusion criteria patient a complicated with other disease as Diabetes, Arthritis, Blood pressure, kidney disorder and Heart diseases B. Forti eight specimen were collected from an apparently stable control group with respiratory diseases.

The result of Elisa shows high concentration of Interlocken 13 in Tuberculosis (TB) (14.5 ± 0.8bg) and Low concentration in Asthma (9.0 ± 1.7bg)Compare with control (9.3 ± 1.4bg), Microribonucleic acid (625 gene) show higher. gene expression in tuberculosis (9.15 ±1.1)، and lower gene expression showed in Sensitive Bronchitis (2.03 ± 0.4) Compare with control (420 ± 0.2).

In the study of (151 gene) showed higher gene in obstructive pulmonary disease (COPD) (35.32±1.1) and lower gene expression show in sensitive Bronchitis (4.26 ± 2.43) Compare with control (1.64 ± 0.5). higher Percent of Bacteria is *streptococcus pneumonia* and low percent of Bacteria is *klebsiella pneumonia* This study concluded that present correlation between IL-13, MiRNA (625,151) with respiratory disorder.as well when relationship between miRNA(625,151)and IL-13 is positive can use miRNA as abiomarker but when relationship between miRNA(625,151) is negative can use miRNA as adrug.

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Abbreviations	Terms
AHR	airway hyperreactivity
ARDS	acute respiratory distress syndrome
CLCA1	calcium-activated chloride channel regulator
COPD	Chronic Obstructive pulmonary
CRP	C-reactive protein
DNA	deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
G-	Gram-negative bacteria
HLA	human leukocyte antigens
ICS	inhaled corticosteroids
IL	interleukin
IPF	idiopathic pulmonary fibrosis
JAK1	Janus kinase 1
MDR	multidrug-resistant
miR	microRNAs
NK	natural killer
qPCR	quantitative polymerase chain reaction
RSV	viruses or respiratory syncytial
SARS	severe acute respiratory syndrome
SERPINB2	serpin peptidase inhibitor, clade B, member 2
STAT6	signal transducer and activator of transcription
TB	Tuberculosis
TLRs	Toll-like receptors
Tyk-2	tyrosine kinase 2
WBC	white blood cell
WHO	World Health Organization
RSV	respiratory syncytial virus
TSLP	thymic stromal lymphopoietin
IPF	idiopathic pulmonary fibrosis

1-1 Introduction

Respiratory disorder is susceptible to two different diseases. Common colds, sinusitis, pharyngitis, epiglottitis, and laryngotracheitis are examples of upper respiratory infections. Lower respiratory system infections include bronchitis, bronchiolitis, and pneumonia (Lv *et al*., 2023)**.**

The cause of the majority of upper respiratory infections is a virus, bacterial and environmental causes. The outliers are epiglottitis and laryngotracheitis, with severe instances most likely brought on by *Haemophilus influenzae* type b. Pharyngitis caused occurs by *Streptococcoccus pyogenes* frequently. Common colds can typically be identified clinically, according to microbiologic diagnosis. Pharyngitis, epiglottitis, and laryngotracheitis are treated with bacterial and virus cultures of throat swab tissues. Epiglottitis patients also require the collection of blood samples (Scannapieco *et al*., 2022).

Germs or viruses can cause lower respiratory illnesses. Viruses bring on most instances of bronchitis and bronchiolitis. *Streptococcus pneumoniae* is the most frequent bacterium caused by community-acquired *pneumonia*. *Mycoplasma* pneumoniae, Chlamydia spp., Legionella, *Coxiella burnetti*, and viruses are just a few of the pathogens that can cause atypical pneumonia (Gentilotti *et al*., 2022). The pathogenesis of nosocomial pneumonia and pneumonia in immunosuppressed individuals is complex, with *staphylococci* and gram-negative bacteria predominating. Sputum samples are grown for bacteria, fungi, and viruses as part of the microbiologic diagnosis. Infants with bronchiolitis can typically be treated with nasal cleaning cultures. Blood samples and serologic techniques are used for viruses, rickettsia, fungi, and numerous bacteria. Both viral

antigens and antibodies can be detected using enzyme-linked *ELISA* techniques. A quick diagnostic can be made using a DNA probe or polymerase chain reaction to identify unique nucleotide segments for the relevant microbial protein (Georgakopoulou *et al*., 2023).

The detection of immunological parameters, such as white blood cell (WBC) count, can provide valuable information about the immune system's response to an infection, inflammation, or other medical conditions making them useful in assessing the risk of cardiovascular diseases (Plebani, 2023)..

WBCs are a crucial part of the immune system and fight infections and foreign substances. WBC count is determined by counting the number of white blood cells in a blood sample. This can be done using an automated hematology analyzer. Other tests, such as differential WBC count, specific antibody tests, and cytokine analysis, may also be used to provide a more comprehensive assessment of the immune system's status. The interpretation of these parameters should always be done in conjunction with a thorough clinical evaluation by a healthcare professional (Clark and Kruse., 1990).

IL-13 is homologous to IL-4. The biological actions of IL-13 on mononuclear phagocytic cells, capillary cells, epithelium cells, and B cells are similar to those of IL-4. So, IgE isotype switching and VCAM-1 expression are induced by IL-13. The different cellular origins of IL-4 and IL-13's biological actions serve as other markers of distinction. Through this hormonal process, IL-13 causes goblet cells to supplant epithelial cells in the airways, leading to the distinctive airway metaplasia of asthma. It also increases mucus production and generalized airway hyperreactivity

(AHR). The effectiveness of IL-13-targeting treatments in this endotype supports the significance of IL-13 in asthma symptoms accompanied by a strong IL-13 signature (Li ***et al.,*** 2023)

MicroRNA (miRNA) is a class of small, non-coding RNA molecules found in plants, animals, and some viruses. They play a significant role in the regulation of gene expression. Here are some key points about miRNA. miR-151 and miR-625 are two specific microRNAs (miRNAs) that are part of a larger class of small non-coding RNA molecules. miRNAs are important regulators of gene expression and play a role in various biological processes, including development, differentiation, and disease (Lee ***et al***., 2022; & Koprivec and Majdič., 2023)

microRNAs are small RNA molecules that play a crucial role in the regulation of gene expression, and they have broad implications in both normal physiological processes and various disease states. miR-151 is a microRNA that has been involved in regulating various cellular processes, including cell proliferation, invasion, and metastasis. Researchers have identified specific target genes that are regulated by miR-151, and these genes are involved in pathways related to cancer progression (Burdiel *et al.*, 2023).

MiRNA-625 may contribute to asthma pathogenesis, according to research. In asthmatics' airways, miRNA625 expression is abnormal. In asthma, miRNA-625 may regulate immune response and inflammation. Tuberculosis: MiRNA625 may be a biomarker. Blood, sputum, and lung tissues of tuberculosis patients express miRNA-625 differently. MiRNA-625 may help diagnose and prognose TB. COPD: MiRNA-625 is linked to COPD development and progression (Tan *et al*., 2022). COPD patients had

altered miRNA-625 expression, which may modulate inflammation, oxidative stress, and tissue remodeling. The role of miRNA-625 in bronchitis is less well-studied than in other respiratory illnesses. However, recent study suggests that miRNA-625 dysregulation in bronchitis patients may cause airway inflammation and tissue damage. A growing body of data links miRNA-625 to COVID-19. COVID-19 patients exhibit altered miRNA-625 expression, which may regulate immunological responses and viral replication in SARS-CoV-2 infection. MIR-625-5p was considerably down-regulated in children with dust mite-induced asthma relative to controls, but Cbl proto-oncogene transcript levels were. These miRNAs may regulate immune response and inflammatory cytokine pathways.(Dong *et al*., 2016).

1-2 Aims of the Study

1 -To investigate the expression levels of microRNA-625 and microRNA-151 in respiratory disorders, such as asthma, chronic obstructive pulmonary disease (COPD), and peumonia, and to correlate their expression with IL-13 levels.

2 -To investigate the potential of microRNA-625 and microRNA-151 as targets for the development of novel therapeutic agents for respiratory disorders

3 -Investigate the expression levels of IL-13 in different respiratory disorders، such as asthma, chronic obstructive pulmonary disease (COPD), Tuberculosis, sensitive bronchitis and bronchiolitis.

4 -Investigate the expression levels of microRNA625 in respiratory disorders and its relationship with IL-13 levels.

5 -Determine whether microRNA625 can regulate IL-13 expression or vice versa.

6 -Investigate the effects of microbial infections, such as viral or bacterial infections, on IL-13 expression in respiratory cells.

2. Literature Review

2.1 Respiratory Disorder

Respiratory disorders refer to a wide range of conditions that affect the Upper and lower respiratory system. These disorders can range from mild to severe and can affect people of all ages. Some of the most common respiratory disorders include:

2.1.1 Asthma

Asthma is a chronic condition that causes the airways to become inflamed and narrow, making it difficult to breathe. Symptoms include wheezing, coughing, chest tightness, and shortness of breath (Thomas *et al*., 2022)

Asthma is one of the most common diseases in the world, affecting about 300 million people worldwide, and it affects the respiratory system. It causes narrowing and obstruction of the airways, making it difficult for the patient to breathe, and it gets worse at times when the asthmatic is exposed to triggers, such as dust, pets, pollen and small particles in the air (Rattu *et al*., 2023).

The exact causes of asthma are unknown so far, but experts believe that there are several factors that may play a role in the development of asthma, and these factors include: exposure to environmental pollution, smoke, and small particles in the air and strong chemical odors, exposure to viral infections, and exposure to allergies to different foods and things (Gauvreau *et la*., 2023).

Asthma is characterized by several symptoms, including: a dry cough, a feeling of tightness or pain in the chest, a feeling of discomfort, wheezing or wheezing when breathing, and difficulty breathing at night or early in the morning (Wallace-Farquharson *et al*., 2022).

Asthma treatment methods vary according to the type and severity of the disease, and treatment may include various medications such as nebulizers, anti-inflammatory drugs, bronchodilators, and anti-allergic drugs. Patients can also learn healthy breathing techniques (Rodriguez *et al*., 2022).

2.1.2 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease is a progressive lung disease that causes breathing difficulties. It includes chronic bronchitis and emphysema, which cause chronic coughing and shortness of breath (Celli *et al*., 2022).

Chronic obstructive pulmonary disease is a chronic medical condition that affects the lungs and leads to obstruction of the airways, and it can lead to difficulty breathing and becomes worse with time, and a person may develop this disease as a result of exposure to smoking, air pollution, dust, chemicals materials, and germs. Symptoms of chronic obstructive pulmonary disease include chronic cough, persistent mucus, shortness of breath, body fatigue, chest expansion, and blue-gray skin (Kim *et al*., 2023).

To treat this disease, patients are advised to get rid of risk factors, such as quitting smoking, avoiding exposure to pollution, taking medications prescribed by the attending physician, resorting to physical therapy, and physical exercise (Adeloye *et al*., 2022).

Chronic obstructive pulmonary disease can also be prevented by maintaining a healthy diet, exercising regularly, avoiding exposure to chemicals and dust, maintaining a healthy lifestyle, and recognizing the signs and symptoms of the disease to reduce the risk of infection (Poto *et al*., 2022).

2.1.3 Pneumonia:

Pneumonia is an infection that inflames the air sacs in one or both lungs, causing coughing, fever, chest pain, and difficulty breathing (Gereige and Laufer., 2013).

Pneumonia is known as inflammation of the lungs, and it is a medical condition that occurs as a result of acute inflammation of the lung tissue, which leads to damage and obstruction of the breathing process. Pneumonia is a serious disease and, in rare cases, may lead to death (Scannapieco *et al*., 2022).

There are many causes that may lead to pneumonia, including exposure to Microorganism, air pollution and smoke as well as smoking, and chronic diseases such as diabetes, heart disease, and chronic respiratory disease contributed to pneumonia. The symptoms of pneumonia are fever, dry cough or yellow or green sputum, difficulty breathing, chest pain, extreme fatigue, and in some rare cases this can be accompanied by headache and nausea (Klompas *et al*., 2022).

Treatments for pneumonia depend on the cause of infection and include antibacterial, antiviral, and antifungal drug treatments, German therapy and analgesics to relieve pain and fever, and chemotherapy in some serious cases. Patients should avoid smoking, exposure to air pollution, fluid intake, and adequate rest to speed up recovery. To avoid pneumonia, must avoid smoking, exposure to air pollution, maintain personal hygiene, eat healthy food, and exercise regularly (Fan *et al*., 2023).

2.1.4 Tuberculosis:

Tuberculosis (TB) is a bacterial infection that affects the lungs and can be spread through the air (Kherabi *et al*., 2022).

Tuberculosis is an infectious disease caused by bacteria called *Mycobacterium* tuberculosis. TB affects the lungs in most cases but can affect any other part of the body such as the kidneys, bones and lymph nodes. Tuberculosis is transmitted through the air, as the bacteria that cause the disease are inhaled from a person with tuberculosis who carries the germ in lungs (Paton *et al*., 2023).

Symptoms of TB are chronic cough, weight loss, night sweats, shortness of breath, fever, and loss of appetite. In some cases, tuberculosis patients may not show any symptoms. People with tuberculosis should get immediate medical treatment to avoid serious complications (Gunasekera *et al*, 2022).

Treatment for tuberculosis involves taking antibiotics for a long time, usually 6-9 months, until the germs that cause the disease are killed. The patient may need to receive medical treatment in the hospital in cases of serious injury or if home treatment does not respond (Dartois and Rubin., 2022).

Tuberculosis can be prevented by avoiding exposure to the germs that cause the disease, such as avoiding contact with people who have TB and covering the mouth and nose when coughing or sneezing. Tuberculosis prevention can also be achieved by immunizing children with anti-tuberculosis vaccines (BCG), which should be given to children in areas with high rates of tuberculosis (Falzon *et al*, 2022).

2.1.5 Bronchitis

Bronchitis is a common respiratory condition in which swelling and inflammation occur in the airways that carry air to the lungs. Bronchitis can be acute or chronic and is generally diagnosed through symptoms and various medical tests. Common bronchitis symptoms include coughing, phlegm, difficulty breathing, and tension and tightness in the chest. Bronchitis could be a sign of another respiratory health problem, such as asthma, sinusitis, or pneumonia (Wenzel and Fowler., 2006).

Diagnosing bronchitis requires a physical exam, a comprehensive evaluation of symptoms, a medical history, and medical tests, such as laboratory tests and medical imaging. Various treatment options are available for bronchitis, including drug therapies, bronchodilator sprays, lifestyle changes, and the prevention of irritants (Cortes-Borrego., 2023).

Treatment options for bronchitis vary according to its type and severity and include pharmacological treatments such as antibiotics, non-steroidal anti-inflammatory drugs, and bronchodilator sprays. Symptoms can also be alleviated by exercising regularly, maintaining a healthy lifestyle, and avoiding exposure to air pollution (Brister *et al*., 2023).

Bronchitis can be avoided by adopting a healthy lifestyle, avoiding air pollutants, and smoking. In addition, avoiding exposure to irritating chemicals is recommended, such as avoiding cold and dry winds, drinking plenty of fluids, and eating foods rich in healthy nutrients such as fruits and vegetables. It is advised to see a doctor if symptoms persist or worsen. (Chandrasekar *et al*., 2023)

2.1.6 Bronchiolitis

Bronchiolitis is a common disease of the respiratory system, and it is a health condition that affects the bronchioles that pass from the larynx and branch out to the lungs. Bronchitis occurs when the wall of the bronchioles

becomes irritated, causing them to swell and become inflamed. This swelling may cause partial or complete obstruction of the airways (Smyth and Openshaw., 2006).

Coughing, secretions, shortness of breath, a feeling of tightness in the chest, and fatigue characterize symptoms of bronchiolitis. Coughing and secretions may increase at night and when exposed to irritating substances such as smoke, dust, and chemical odors (Silver and Nazif., 2019).

The causes of bronchiolitis vary and can be caused by smoking, exposure to air pollution, various irritating substances, and viral and bacterial infections. People can develop chronic bronchiolitis if exposed to risk factors such as smoking and exposure to air pollution for a long time (Dalziel *et al*., 2022).

Diagnosing bronchiolitis requires a physical exam, a comprehensive assessment of symptoms, a medical history, and medical tests, such as laboratory tests and medical imaging. Various treatment options are available for bronchiolitis, including drug therapies, bronchodilator sprays, lifestyle changes, and the prevention of irritants (Lawrence *et al*., 2022).

2.1.7 Covid 19

COVID-19 is a viral disease caused by the novel coronavirus (SARS-CoV-2) that primarily affects the respiratory system and can cause mild symptoms like fever, cough, and difficulty breathing, or progress to severe cases such as pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure, and even death in rare cases (Msemburi *et al*., 2023).

The virus is primarily spread through respiratory droplets that are released when an infected person talks, coughs, or sneezes, and can also spread through touching surfaces contaminated with the virus and then touching the mouth, nose, or eyes. The disease was first identified in

December 2019 in Wuhan, China, and has since spread rapidly across the globe, leading to a pandemic (Yang *et al*., 2020).

Because of the rapid spread of the coronavirus disease 2019 (COVID-19) and the high fatality rate associated with it, the situation is now being treated as a worldwide emergency. The severe acute respiratory syndrome coronavirus, also known as SARS-CoV2, is the etiological agent of COVID-19, and its occurrence across the world is rapidly increasing. Patients with COVID-19 are at risk for developing a variety of serious medical conditions, including pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS), and failure of multiple organs. The expanding body of evidence suggests that immunological patterns play a significant role in the development of illness in persons who have been infected with viruses (Hui., 2023).

Patients who are diagnosed with severe acute respiratory syndrome (SARS) have a unique characteristic that is referred to as a drop in peripheral T cell subsets. Because it may be used to swiftly restore peripheral T cell subsets in patients who have recovered from SARS, the number of peripheral T cells can serve as a valid diagnostic test for the disease (Hui., 2023). Another study that revealed that SARS affected the immune system also described a similar occurrence. In a different investigation, it was shown that Ebola patients had less natural killer (NK) cells than healthy donors. Patients who had recovered from the Ebola virus disease displayed low levels of proinflammatory cytokines, but with the onset of Ebola virus sickness symptoms, proinflammatory cytokines rose to higher levels. Since the connection between immune responses and the illness has been discovered (Chow *et al*., 2023), immune characteristics are now recognized as viable indicators for disease progression as well as

potential therapy targets for COVID-19. This is due to the fact that immune characteristics have been shown to be associated with COVID-19.

Immunopathology related to COVID-19. In COVID-19 patients who are extremely unwell and in critical condition, it has been proven that SARS-CoV-2 modifies usual immune responses, hence weakening the immune system and producing uncontrolled inflammatory reactions. These people have anomalies in their granulocytes and monocytes, as well as lymphopenia, lymphocyte activation and malfunction, high cytokine levels, and an increase in both total antibodies and immunoglobulin G (IgG) (Chow *et al*., 2023). Lymphopenia is a common symptom seen in patients diagnosed with COVID-19, particularly in more severe cases. Lymphopenia, which is more likely to be present in those who have severe COVID-19, is one of the most important predictors of patients who have severe disease. In addition, patients show a significant decrease in the number of CD4+ T cells, CD8+ T cells, NK cells, and B cells in their bodies. In severe cases, the proportion of lymphocytes was determined to be lower than 20%. Further analysis found that severe patients had a much lower number of T cells, specifically CD8+ T cells, in comparison to mild cases (Hui.. 2023).

2.2 Bacteria and Respiratory Infections

Lower respiratory tract infections were the most lethal communicable disease in 2015, accounting for 3.2 million fatalities worldwide (Vázquez *et al*., 2018). And nearly 7 million deaths until 2023 due to the Coronavirus; according to the statistics of the World Health Organization، Community-acquired pneumonia is the leading bacterial infectious cause of death in children worldwide, and tuberculosis is still one of the top 10 causes of death (WHO, 2023). *Haemophilus influenzae* type b, *Streptococcus pneumoniae (pneumococcus), Streptococcus pyogenes*

(group A Streptococcus), non-typeable *H.influenzae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Moraxella catarrhalis*, and *Klebsiella pneumoniae* are the other bacterial pathogens that cause bacterial pneumonia elderly adults with concomitant conditions frequently get community-acquired pneumonia due to pneumococcus (Torres *et al*., 2018). On the other hand, multidrug-resistant (MDR) Gram-negative bacteria (GE-) are increasingly the bacteriologic cause of hospital-acquired pneumonia and ventilator-associated pneumonia, two of the most common nosocomial infections globally (Kidd *et al*., 2018).

Streptococcus pneumoniae is a common cause of pneumonia, particularly in children and the elderly. *Haemophilus influenzae* it can cause pneumonia, bronchitis, and sinus infections, especially in individuals with weakened immune systems. *Legionella pneumophila* causes Legionnaires' disease, a severe form of pneumonia. *Mycoplasma pneumoniae* it is responsible for a type of pneumonia called atypical, walking pneumonia. *Bordetella pertussis* it causes pertussis, also known as whooping cough, a highly contagious respiratory infection. *Staphylococcus aureus* can lead to pneumonia, especially in healthcare settings or individuals with weakened immune systems. *Streptococcus pyogenes* can cause strep throat, tonsillitis, and scarlet fever, which can involve respiratory symptoms; these bacteria can be transmitted through respiratory droplets when an infected individual coughs, sneezes, or talks. They can also spread through close contact with contaminated surfaces or objects. Risk factors for respiratory bacterial infections include a weakened immune system, smoking, crowded living conditions, and certain medical conditions (Vázquez *et al*.,2018).

The symptoms of respiratory bacterial infections vary depending on the specific bacterium and the type of infection. Common symptoms may

include cough, fever, chest pain, shortness of breath, fatigue, sore throat, and nasal congestion. Diagnosing respiratory bacterial infections usually involves a combination of medical history, physical examination, and laboratory tests such as sputum culture, blood tests, and chest X-rays (Kidd *et al*., 2018). Treatment typically involves antibiotics targeted at the specific bacterium causing the infection. It's important to note that respiratory infections can also be caused by viruses, such as influenza viruses or respiratory syncytial virus (RSV). Proper diagnosis is necessary to determine the cause of the infection and prescribe appropriate treatment (Horváth *et al*., 2015)

2.3 respiratory system diseases:

Collaboration with clinical and laboratory diagnosis: Using certain laboratories with approved procedures, epidemiology functionally characterizes suspicious and confirmed cases.

Epidemiology offers a record of data and an appropriate collection of samples for predicting the trends in the emergence of infectious and noninfectious diseases.

Impact: Epidemiology discusses mortality, morbidity, life years lost due to death or disability, among other characteristics to quantify the impact of various agents. It also forecasts the propensity of infectious and noninfectious diseases to manifest themselves.

Pathogeny: Epidemiology examines the progression of disease, taking into account its origin, mode of transmission, point of entrance, spread among humans, coping mechanisms, and eradication from the population.

recommending and assessing control strategies, such as resource planning.

Vaccines, education, the environment, biosecurity, chemoprophylaxis, and passive immunization are some of the prevention strategies.

Treatment: This entails using antibiotic, antiviral, and antifungal medications as well as additional supportive techniques like oxygen therapy or mechanical breathing.

2.4 Immunity:

Understanding the connection between genetic variants and the immune response to microbial infections in respiratory illnesses requires immunogenetic evaluation. The immune system is essential for identifying and removing invasive bacteria in respiratory illnesses. However, there are situations when the immune response can become dysregulated, resulting in tissue damage, inflammation, and persistent respiratory conditions (Turner *et al*., 1986).

Numerous genetic variants, including polymorphisms in the genes encoding for cytokines, Toll-like receptors, and human leukocyte antigens (HLA), have been found to influence the immunological response to respiratory infections. These genetic differences may affect the intensity and length of respiratory infections and the emergence of long-term respiratory diseases (Stephenson and Zambon *et al*., 2002).

For instance, polymorphisms in the interleukin-10 (IL-10) gene have been linked to an increased risk of asthma and respiratory infections. Anti-inflammatory cytokine IL-10 is essential for controlling the immune response. Respiratory infections and asthma have been linked to polymorphisms that result in reduced amounts of IL-10 (Chernoff *et al*., 1995).

Toll-like receptors (TLRs) have a similar role in identifying microbial invaders and triggering the immune response. TLR polymorphisms have been linked to a greater propensity for respiratory infections, including pneumonia and TB (Fukata *et al*., 2009).

Respiratory conditions have also been associated with HLA genes, which are essential in presenting antigens to T lymphocytes. For instance, the HLA-DQ gene has been linked to an increased risk of developing asthma and chronic obstructive pulmonary disease (COPD) (Esmaeilzadeh. *et al*., 2015).

Microbial infections can contribute to the emergence and escalation of respiratory illnesses in addition to hereditary causes. Infections caused by bacteria and viruses can set off an immune response that damages tissue in the respiratory system and causes inflammation. Recurrent infections can occasionally bring on chronic respiratory conditions like bronchiectasis and COPD (Kim *et al.*, 2023).

2.4.1 Cytokines

Cytokines are a group of small proteins that are secreted by various cells in the body and lead to communication between these cells and the regulation and activation of the immune system and other bodily functions. Cytokines belong to the family of cytokine proteins (Opal and DePalo. 2000).

Cytokines play an important role in defending the body against diseases and infections. They are secreted when injury, inflammation or infection occurs to help the body deal with these conditions. Cytokines also regulate the immune system and enhance the immune response in viral, bacterial and fungal infections (Ghofrani Nezhad *et al*., 2023).

There are many types of cytokines, each performing different bodily functions. Cytokines are produced by immune cells such as lymphocytes, macrophages, neurons, stem cells, particle cells, and vascular cells (Dinarello., 2007).

Disruption of cytokine production and its interaction with the immune system is one of the main causes of many immune and inflammatory

diseases, such as rheumatoid arthritis, inflammatory bowel disease, asthma, cancer, nervous system diseases, and patients suffering from depression (Hsu *et al*., 2022).

The actions of cytokines are mediated through specific cytokine receptors on the surfaces of target cells. Although cytokines usually affect adjacent cells, they can act at a dis- stance and affect the cells producing the cytokines themselves. Many of these cytokines exhibit pleiotropy and have overlapping functions, making their individual roles in the pathogenesis of asthma and allergic disease challenging to differentiate (Hamid and Tulic., 2009).

2.4.2 Interleukins

Interleukins are a group of small proteins that play an essential role in the body's immune response. Interleukins are cytokines, proteins secreted by immune cells and other cells in the body that communicate between these cells (Mizel., 1989).

Interleukins stimulate the activity of immune cells, control coordination between them, and stimulate cell growth and differentiation. Interleukins also play an essential role in controlling inflammation and the inflammatory immune response. Interleukins are released when an injury, inflammation, or infection occurs, and they trigger inflammation to activate the immune system to fight infection (Peluzzo and Autieri., 2022)

Many types of interleukins are numbered, such as IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, and others. Each type of interleukin performs different functions in the immune system and is secreted in specific tissues in the body and in specific circumstances, as shown in Figure 2.1 (Hsu *et al*., 2022).

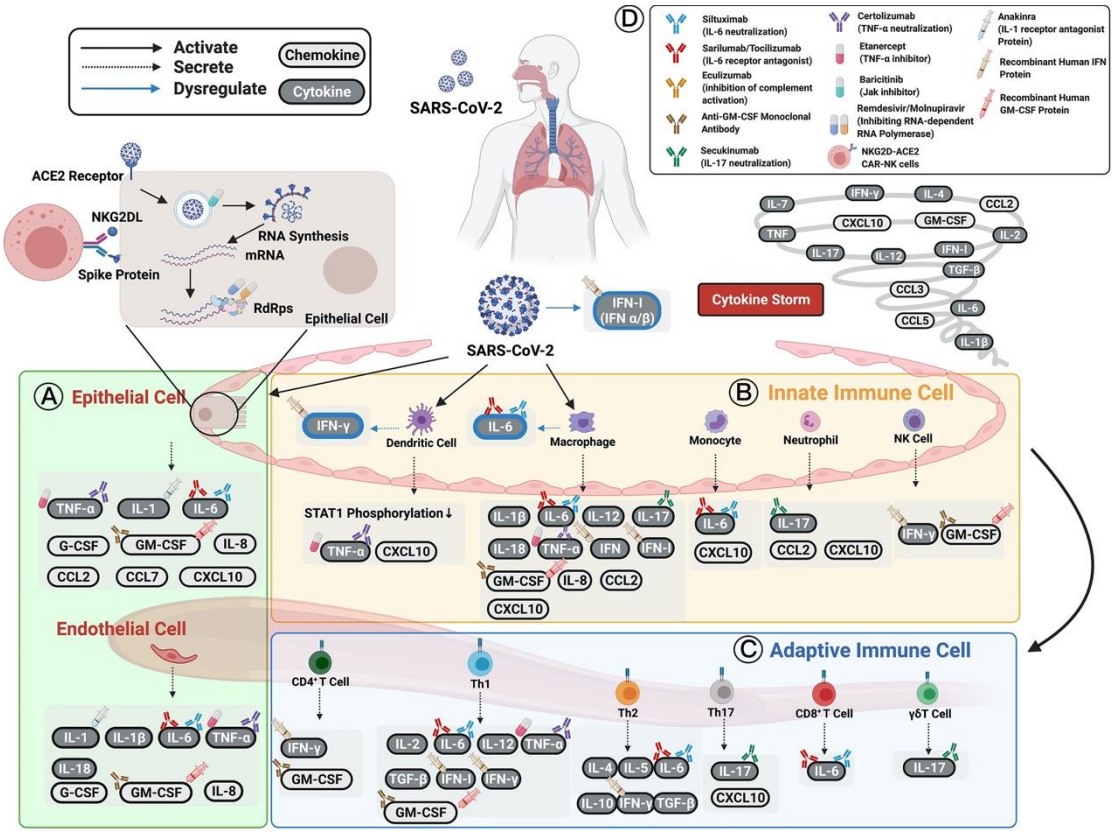


Figure 2.1: types of interleukins (Hsu *et al.*, 2022).

Interleukins play an essential role in many health conditions, such as chronic inflammatory diseases and diseases resulting from a deficiency of the immune system, as shown in Figure 2.1. For this reason, the level of interleukins in the blood and living tissues is analyzed to diagnose, monitor, and treat many pathological conditions (Hsu *et al.*, 2022).

2.4.3 Interleukin-13 (IL13)

Over the course of the last two decades, an increasing number of cytokines have been shown to be associated with the pathophysiology of asthma. Since mechanistic studies have found a relationship between interleukin (IL)-13 and asthma, it has been postulated that inhibiting IL-13 might aid people who are resistant to the medications that are now available (Chiba *et al.*, 2012).

According to the most recent models of allergic asthma, one of the earliest events in the beginning of allergic inflammation is the production of CC family chemokines such as MCP-1 (CCL2), MIP-1 alpha (CCL3), and MIP-1 beta (CCL4), as well as epithelial cytokines such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). These cytokines target resident cells in the airway in order to produce an influx of inflammatory cells from the circulation. In addition to activating and mobilizing dendritic cells, this is accomplished via targeting resident cells in the airway. IL-33 potently activates mast cells, causing them to emit vasoactive amines, lipid mediators, chemokines, and cytokines (Pushparaj et al,.2009). This occurs despite the fact that both IL-25 and IL-33 activate incompletely defined IL-25R+ lymphoid cells in tissues, which produce IL-5 and IL-13 (Hurst *et al*., 2002).

T cells in lymph nodes can either migrate to B cell zones to grow into T follicular helper cells or enter the bloodstream to mature completely Th2 cells and move to the lungs. Both of these pathways result in the T cells leaving the lymph node. TH2 cells in lung tissue are responsible for the secretion of IL-4, IL-5, and IL-13, which are cytokines that enhance the survival and chemotaxis of basophils and eosinophils (Hsu *et al*., 2022).

IL-13 has been designated a critical mediator of asthma due to the fact that it has been demonstrated to independently elicit several of asthma's important physiologic and pathologic characteristics. This is despite the fact that all of the aforementioned cytokines play roles in beginning and maintaining airway inflammation. As was mentioned before, activated CD4+ TH2 cells are the principal source of the 17-kDa glycoprotein known as IL-13. Other sources of this protein include, to a lesser degree, activated TH1 cells, CD8+ T cells, mast cells,

basophils, eosinophils, and natural killer T cells (Krause et al., 2006). IL-13 has a broad variety of effects on asthma, some of which include altering the generation of plasma cell antibodies from IgM to IgE (Punnonen et al., 1993), boosting eosinophil migration into the lung (Horie *et al*., 1997), and upregulating adhesion molecules that bind to eosinophils (Luttmann *et al*., 1996). All of these actions have been linked to asthma. It also induces greater permeability and sloughing of airway epithelial cells, which has been related to reduced production of the structural focal adhesion protein paxillin (Ramirez-Icaza *et al*., 2004).

The IL-13 receptor is a heterodimer complex made up of the IL-13 receptor(R)alpha 1 and the IL-4Ralpha (Skowron-zwarg et al. 2007). This complex is expressed in B cells, monocytes/macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells, and airway smooth muscle cells. As a consequence of this initial attachment of IL-13 to the IL-13Ralpha 1 subunit, the IL-4Ralpha chain is subsequently re-recruited for service. One of the signal transduction pathways that is triggered when IL-13 binds to the IL-13Ralpha 1/IL-4Ralpha complex is the tyrosine kinase proteins Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk-2). Both of these proteins may be present in the cytoplasm and are constitutively linked with IL-13 receptor subunits. Because of the activation of these tyrosine kinase proteins, the signal transducer and activator of transcription 6 (STAT6) is phosphorylated and activated, and it is subsequently delivered to the nucleus (Chiba et al., 2012). IL-13Ralpha 2, one of the additional receptor chains found on the cell surface, does not form a pair with any of the other receptor proteins. According to Zheng et al. (2008), binding of IL-13 to this receptor frequently does not activate

signal transduction proteins. Because of this, it is possible that it plays a critical role as an auto-regulatory factor in suppressing the effects of IL-13. As shown in figure 2.2

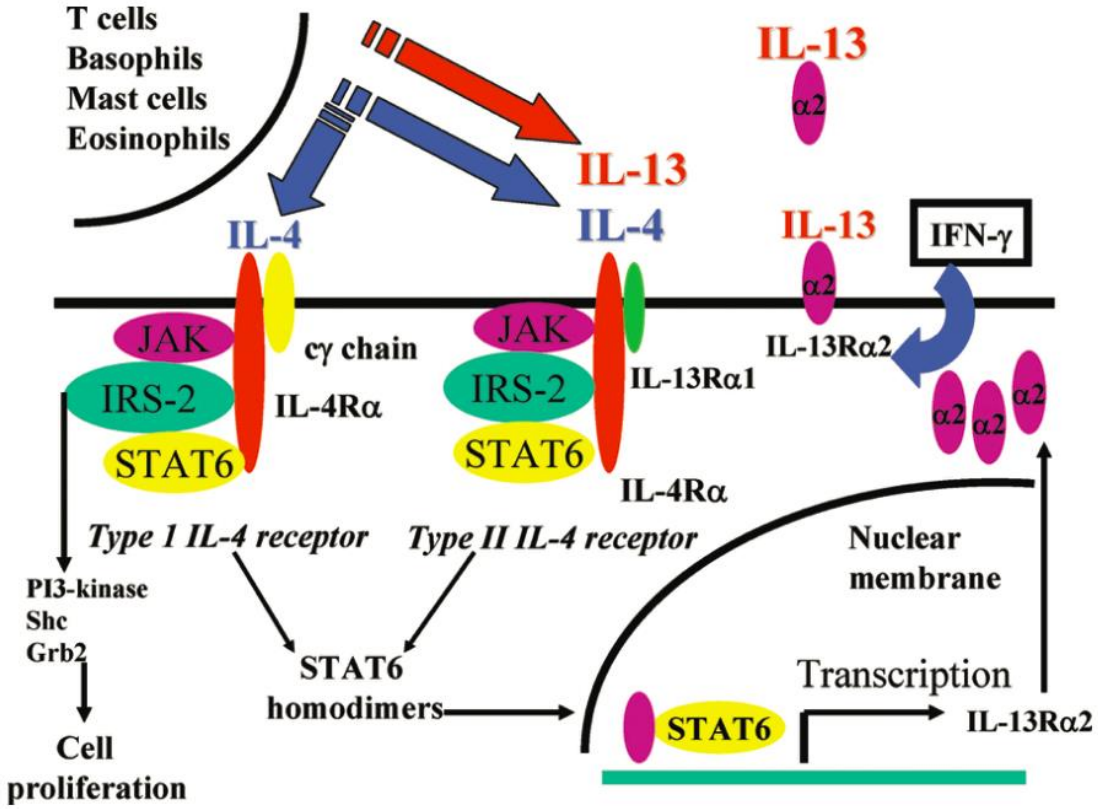


Figure (2.2) Interleukin (IL)-13/IL-4 receptors and signal transduction pathways. (Wills-Karp., 2004)

According to research done in allergic mouse models of the disease, numerous pathophysiologic characteristics of asthma can be induced by IL-13 alone (Grunig et al,.1998). The hypothesis that IL-13 plays a key role in the development of asthma in persons is supported by research that was conducted on patients who already suffer from asthma. Prieto et al. (2000) found that mild allergic asthmatics who participated in segmental allergen challenge tests had an increase in IL-13 protein and mRNA in their bronchoalveolar lavage fluid. This finding suggests that this cytokine may have a role in the pathophysiology of asthma. In addition, IL-13 may have a role in asthma, as shown by research that found a

connection between polymorphisms of IL-13 and its receptors and the occurrence of asthma and bronchial hyperresponsiveness (Howard et al., 2002). This research was conducted on genetic samples (Li et al., 2010) Asthma has been connected to a number of different variations in the RAD50-IL13 region on chromosome 5q31.1, which suggests that the genetic control of this cytokine may contribute to an increased risk of asthma as shown in figure 2.3. (Wills-Karp, 2004)

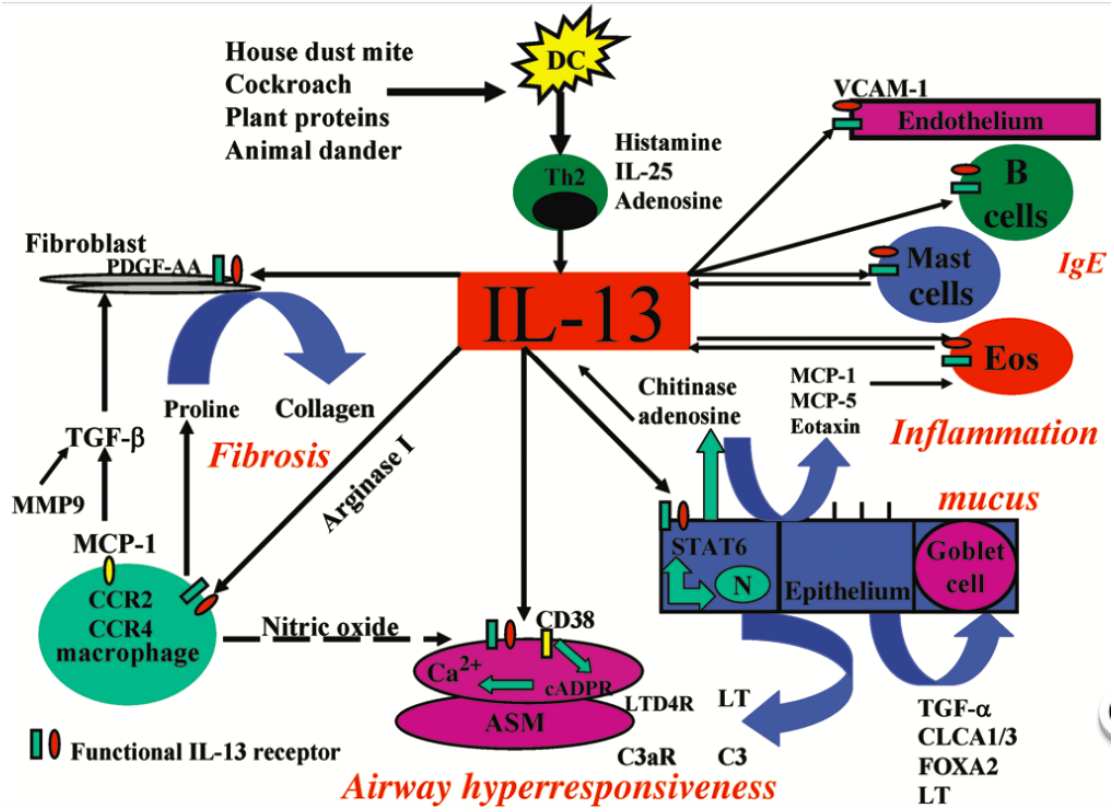


Figure (2.3) Schematic representation of the potential mechanisms by which interleukin (IL)-13-induces the pathophysiological features of asthma. (Wills-Karp., 2004)

According to Simpson et al. (2006) research, TH2 inflammation of the airways is characterized by the presence of activated CD4+ T lym hocytes, eosinophils, and TH2 cytokines such as IL-4, IL-5, and IL-13. Around fifty percent of people who have asthma have a pattern of inflammatory and cytokine production that is

characterized as a TH2 phenotype (Berry *et al.*, 2007). However, this expression pattern is not ubiquitous. Patients with a TH2 phenotype react to therapy with inhaled corticosteroids much better than patients with a non-TH2 phenotype, highlighting the therapeutic importance of heterogeneity in cellular infiltrates and cytokine activity. Patients with a non-TH2 phenotype respond to treatment with inhaled corticosteroids significantly worse than patients with a TH2 phenotype (Simpson et al.,2006) According to Woodruff et al. (2009), many methodologies have been utilized in order to differentiate between the various subgroups of asthmatics. This is because determining a patient's inflammatory phenotype might be therapeutically beneficial.

Eosinophilia in the blood is a common sign associated with asthma, however it is not always present in these people. According to the findings of Woodruff et al., (2009) asthmatics with an enhanced bronchial expression of IL-5 and IL-13 had considerably higher peripheral blood eosinophil counts than non-asthmatic controls did. This was the case when the two factors were compared. Despite the fact that there is a substantial degree of overlap between these two groups, which makes separation difficult (Woodruff et al. 2009), this group of patients also had considerably higher mean blood eosinophil levels than asthmatics who did not have TH2 asthma. When the two variables were tested throughout a range of values in a different research, it was found that there was no significant link between blood and airway eosinophil counts. This was the finding from the comparison of the two variables. In addition, it has been revealed that blood eosinophil counts are very variable, with a correlation value of 0.18 when comparing two samples obtained over a period of three to five weeks. This finding was made possible by the fact that blood eosinophil levels are highly variable (Jia *et al.*, 2012)

Therefore, despite the fact that a high blood eosinophil count may provide some insight into TH2 status, it appears that this test is inconsistent and insensitive, which severely limits its value as a biomarker. Counts of eosinophils in the sputum. In individuals with asthma, sputum eosinophil levels have been demonstrated to fall anywhere along a continuum in a number of different investigations (Jia *et al*., 2012). A significant number of people who suffer from asthma regularly have low eosinophil levels that are equivalent to those seen in healthy patients. These findings are consistent with the findings from bronchoscopy investigations, which similarly identified persons with eosinophilic and non-eosinophilic types of asthma in patients (Wenzel *et al*., 1999).

A cutoff of 2% can be used, according to published reference values for eosinophils in induced sputum from healthy subjects, to determine whether sputum eosinophilia is present or absent; in other words, subjects with 2% or more sputum eosinophils have the condition, and subjects with less than 2% do not have the condition (Lee, 2014). Sputum eosinophilia, defined as 2% eosinophils, was discovered in 36% of participants who had never used inhaled corticosteroids (ICS), and in 17% of individuals who had received ICS therapy in a cohort of 995 adult asthmatics (Hastie, et al.2017). The definition of sputum eosinophilia was based on the presence of 2% eosinophils. According to a longitudinal subgroup analysis conducted during this trial, 22% of ICS-naive patients had persistent sputum eosinophilia, 31% had intermittent eosinophilia, and 47% did not have any eosinophilia in their sputum at all. Studying 81 asthmatics and 32 control individuals, Saha et al. (2008) examined sputum samples and performed bronchoscopic biopsies in order to determine whether or not sputum eosinophilia was connected with pulmonary IL-13 activity (Spanevello A, *et al*., 2000). Because there is a positive link between the presence of

sputum eosinophilia and the expression of IL-13 in the bronchial submucosa, sputum eosinophil counts have the potential to be employed as a measurement of TH2 inflammation.

Since IL-4 and IL-13 control the synthesis of IgE and total blood IgE is connected to an increased risk of acquiring asthma, total IgE is a possible biomarker for defining the phenotype of asthma. This is because IL-4 and IL-13 govern the production of IgE. Serum IgE was found to have a modest correlation with eosinophilic inflammation, making it the least sensitive of many possible indications, including exhaled serum periostin, nitric oxide, and blood eosinophils (Jia *et al*., 2012). Other possible indicators include exhaled serum periostin(Kuiper, *et al*., 2006).

proteins that coat the inner surfaces of the airways contributed in Th2 activation can be measure In the current study, researchers are seeking for proteins that are produced from the bronchi and are associated to TH2 airway inflammation. These proteins have the potential to act as a replacement for TH2 cytokine markers. A recent study looked at the expression of three genes that are regulated by IL-13 in the airway epithelial brushings of mild asthmatics who have never been treated with corticosteroids. These genes are periostin (POSTN), calcium-activated chloride channel regulator 1 (CLCA1), and serpin peptidase inhibitor, clade B, member 2 (SERPINB2) (Lee., 2014).

According to Takayama et al. (2006), periostin, in particular, plays an important part in the biology of the airways since it may have both autocrine and paracrine effects on fibroblasts and epithelial cells, both of which have the ability to remodel the airways. In a research that used bronchoscopic sampling, almost half of the patients were found to have higher levels of one or more of these proteins in the epithelial tissue of their airways. According to Jia et al., (2012) research, the levels of eosinophil,

mast cell IL-5 and IL-13 mRNA found in bronchial tissue were related with these protein quantities. Patients who had greater levels of TH2-associated proteins reacted better to the inhaled ICS therapy than those who were in the non-TH2 group. This finding is noteworthy.

Nitric oxide, also known as FeNO, is a gas that is exhaled by the tissues of the body, notably the lungs. It has been proven that a moderate correlation exists between eosinophils and FeNO in bronchial biopsies, induced sputum (blood), and bronchoalveolar lavage fluid), among other samples (Warke *et al*., 2002). According to Chibana et al. (2008), the enzyme NO synthase, which is responsible for the production of NO, is directly controlled by IL-13. Elevated FeNO has also been shown to be a more reliable indicator of the chance of steroid responsiveness than spirometry, bronchodilator response, peak flow variation, or airway hyperresponsiveness to inhaled methacholine (Smith et al., 2005). This was proved by the fact that elevated FeNO was able to predict the likelihood of steroid responsiveness. An increased level of FeNO is indicative of increased IL-13 activity as well as TH2 inflammation. While previous researchers have observed steroid responsiveness at levels as low as 33 part per billion, there is correlation between increases in FeNO levels and improvements in FEV1 after treatment with ICS. According to the findings of Smith et al. (2005), the amount of NO that provided the most accurate discrimination was 47 ppb. Levels lower than 22 ppb were necessary to make an accurate prediction of the chance of successfully halting ICS. In general, a low level of FeNO is more likely to rule out the presence of airway eosinophilia than a high level of FeNO is to forecast its presence. Because the majority of the data came from patients with mild to moderate asthma, it is unclear whether or not these findings may be extrapolated to

asthmatic populations with more severe symptoms (Zacharasiewicz *et al*., 2005).

2.5 Micro RNA

post-transcriptional silencing of target genes is controlled by a class of small RNA molecules known as microRNAs (miRNAs). These miRNAs range in length from 19 to 25 nucleotides. A single microRNA can target hundreds of different mRNAs and can have an effect on the expression of several genes, many of which are involved in a biological pathway that is functionally interrelated. Asthma, eosinophilic esophagitis, allergic rhinitis, and eczema are only few of the allergic conditions that have been shown to be regulated by miRNAs (Zhang X *et al*., 2014).

Small endogenous RNAs termed microRNAs (miRNAs) govern post-transcriptional gene expression. Growing attention is being paid to miRNAs in the field of allergy research since these little RNA molecules are critical for controlling how genes are expressed and provide promising prospects for the development of biomarkers. Both small interfering RNA (miRNA) inhibitors and small interfering RNA (miRNA) mimics have shown promise as possible novel medicines in preclinical research (Cui, et al, 2021).. Several different technological platforms have been developed in order to perform tasks such as the separation of miRNA, the quantification of miRNA, the profiling of miRNA, the discovery of miRNA targets, and the modification of miRNA levels in vitro and in vivo. In this section, we will examine the primary technological platforms while taking into account the merits and downsides of each (Cirera *et al*., 2011).

suggest reviewing references 6 through 9 for further information on the biology of miRNAs (Jonas and Izaurralde., 2015).

2.5.1 miRNA expression detection

Expression of microRNA can occur in both tissue samples and cell-free body fluids like serum or plasma. Tissue samples are more likely to include more cells. RNA sequencing, in-situ hybridization, microarrays, and quantitative polymerase chain reaction (qPCR) are some of the approaches that are being utilized to locate microRNAs. Because the typical length of a miRNA is just 21 to 23 base pairs, the construction of PCR primers is technically challenging due to the fact that the typical length of a PCR primer is around 20 base pairs (Pritchard *et al.*, 2012). The solution is to use a miRNA-specific stem loop primer for transcription (Figure 2.4) or universal reverse transcription by adding a 3′poly A tail to the miRNA and then using a poly T primer with a universal sequence appended at the 3′ end for reverse transcription (Figure 2.5) to lengthen the miRNA while performing the reverse transcription step. Both of these solutions are shown in Figures 2.4 and 2.5, respectively. After that, qPCR is performed with forward primers and probes that are specific to each miRNA, as well as a reverse primer that is complementary to the stem-loop or the common sequence of the poly T primer (Shi and Chiang., 2005).

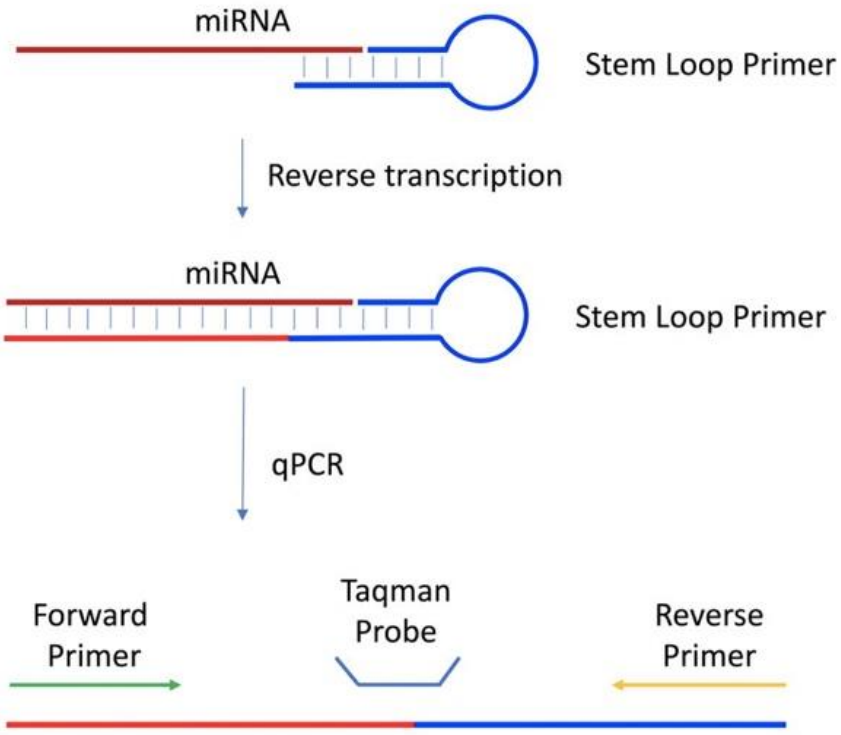


Figure 2.4: qPCR utilizing a miRNA-specific probe, a miRNA-specific forward primer (Green), and a reverse primer complementary to the stem-loop sequence (Orange) is performed after reverse transcription using a miRNA-specific stem-loop primer (Lu *et al*.,2018).

In addition, a universal adapter can be added to the 5' end in order to give an optional universal pre-amplification prior to qPCR (Figure 2.7). This can be done in order to detect targets with primer/probe combinations that are present in extremely low abundances. The stem-loop primer based method, which can only reverse transcribe one miRNA at a time, has a higher level of specificity than the global reverse transcription approach, which has a lower level of specificity.To get around this constraint, multiplex stem-loop primer pools are available (Le *et al*., 2014). Although it is still difficult to separate miRNAs that differ by only 1-2 nucleotides using PCR, qPCR of a specific template will frequently provide 10 to 100-

fold greater amplification signal than a template that only differs by a single nucleotide (Cirera *et al.*, 2011). This is because qPCR amplifies the template more thoroughly.

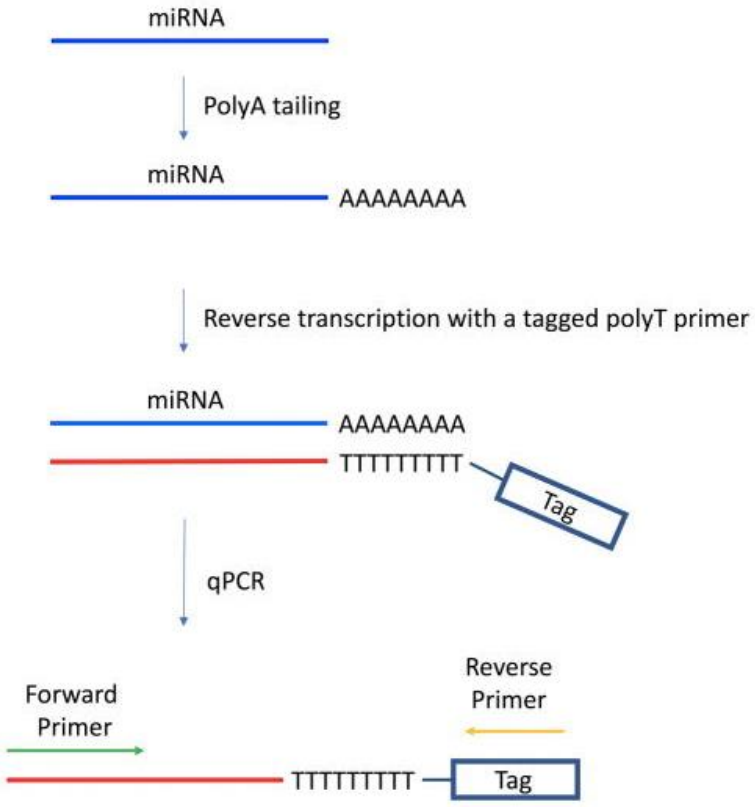


Figure 2.5: Reversetranscription using a Poly T primer with a universal sequence (Tag) added at the 3′end, followed by reverse transcription using polyA tails added to the 3′end of the miRNA, and finally qPCR using miRNA-specific forward primer (Green) and universal reverse primer (Orange) (Lu *et al.*,2018).

Multiplex qPCR-based arrays and hybridization-based arrays are two examples of microarray-based techniques. The pre-plated PCR primer/probes used in the qPCR microarrays are dispersed throughout 96 or 384 well plates. A microfluidic card is available for low-input material that needs as little as 1 ng of total RNA, and microfluidic devices are available that allow single-cell miRNA profiling (Pritchard *et al.*, 2012)

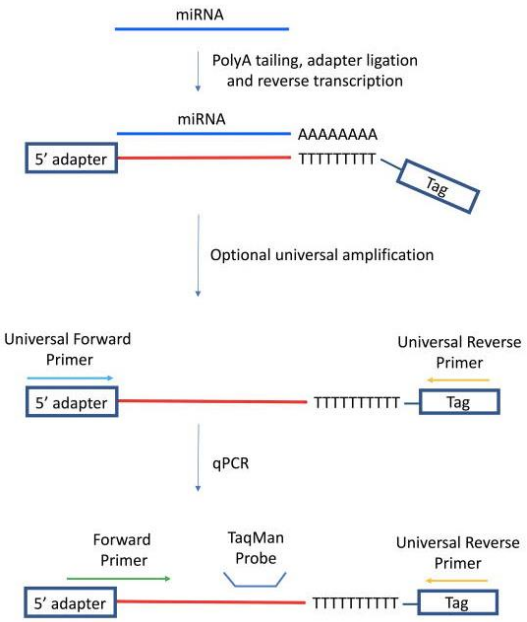


Figure 2.6: Reverse transcription with a Poly T primer that has a universal sequence (Tag) appended at the 3′end, followed by reverse transcription with a universal amplification step that adds a 5′adapter sequence and polyA tails to the 3′end of the miRNA, followed by qPCR with a miRNA specific probe, a miRNA specific forward primer (Green), and a universal reverse transcription that does the same (Lu *et al*.,2018).

The benefit of in-situ hybridization is that it may reveal the tissue from where the target miRNA originated and the relative expression levels in other tissue compartments. Locked nucleic acid probes are commonly employed to improve mismatch discrimination and binding affinity, (Nielsen., 2012) A small RNA sequencing library may be built and processed using high throughput sequencing, allowing for the quantitative identification of every small RNA species present in a given sample and the discovery of new miRNAs and other small non-coding RNAs (Pritchard et al., 2012). The library must be built from a starting material of 10 to 50 ng of tiny RNA.

2.5.2 microRNA-151

MicroRNA-151 (miRNA-151) is a type of small non-coding RNA molecule that regulates gene expression by binding to specific messenger RNA (mRNA) molecules, causing them to be degraded or preventing them from being translated into proteins. miRNA-151 has been identified in many different species, including humans, and has been found to play a role in various biological processes, including cell proliferation, differentiation and apoptosis As shown in figure (2.7). (Kawahara *et al*., 2007).

Understanding the function of miRNA-151 and other microRNAs is an important area of research in molecular biology, as these molecules may serve as potential targets for the development of new therapies for various diseases.

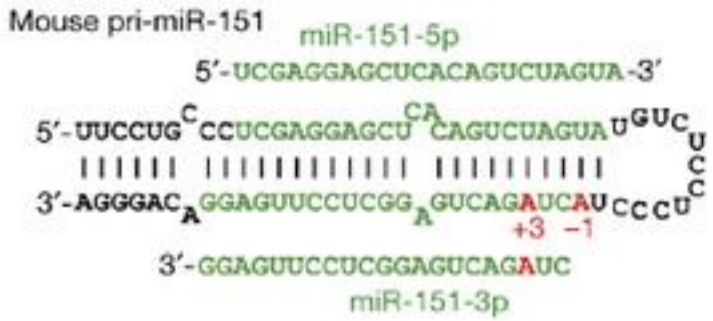


Figure (2.7) RNA editing of pri-miR-151 RNA. The two editing sites of mouse pri-miR-151 are shown in red. The regions known to be processed into the mature miR-151-5p and miR-151-3p are shown in green. An individual editing site is indicated by a number, with the 5′ end of the mature miR-151-3p sequence counted as +1.

2.5.3 MicroRNA-625

A short RNA molecule is what molecular biologists refer to as mir-625 microRNA. The role of microRNAs is to influence the expression levels of other genes through a variety of different ways. According to (Qu et al. 2023), a large number of microRNAs have crucial roles in the genesis and progression of cancer. There is limited research on the specific role of miRNA-625 in respiratory disorders, but some studies have suggested that this molecule may be involved in the development and progression of certain respiratory diseases (Cui *et al*., 2021).

For example, a study published in the journal BMC Pulmonary Medicine found that miRNA-625 was significantly upregulated in patients with chronic obstructive pulmonary disease (COPD), a common respiratory disorder characterized by persistent airflow limitation. The researchers also found that miRNA-625 was associated with decreased expression of the gene TGFBI, which has been implicated in the pathogenesis of COPD (Tan *et al*., 2019).

Another study published in the Journal of Cellular Biochemistry found that miRNA-625 was upregulated in lung tissue from patients with idiopathic pulmonary fibrosis (IPF), a progressive and fatal lung disease characterized by the formation of scar tissue in the lungs. The researchers also found that miRNA-625 was involved in the regulation of the gene HSPB1, which has been implicated in the development of IPF (Qu *et al*., 2023).

While more research is needed to fully understand the role of miRNA-625 in respiratory disorders, these findings suggest that this molecule may play a role in the pathogenesis of certain respiratory diseases and could potentially serve as a target for the development of new therapies (Tan *et al*., 2019).

2.5.4 housekeeping miRNAs

The most frequent strategy for normalizing RT-qPCR data for miRNA expression is to employ housekeeping genes as an endogenous control. Housekeeping genes are also known as reference genes. According to Abd-El-Fattah et al. (2013), housekeeping genes are internal reaction controls that are utilized in the process of gene expression normalization for various miRNAs. Housekeeping genes can have many isoforms. Housekeeping miRNAs refer to a group of microRNAs that are commonly expressed at relatively stable levels across different tissues and under different conditions. They are frequently used as internal controls for quantifying other miRNAs in various biological samples (Hubert *et al*., 2023).

In the context of respiratory disorders, several studies have identified housekeeping miRNAs that may be useful as reference genes for the normalization of miRNA expression levels in respiratory tissues. For example, a study published in the Journal of Thoracic Disease found that miR-16, miR-103, and let-7a were stable housekeeping miRNAs in human lung tissues from patients with chronic obstructive pulmonary disease (COPD). Similarly, a study published in Respiratory Research identified miR-16, miR-26a, and miR-103 as stable housekeeping miRNAs in human lung tissues from patients with idiopathic pulmonary fibrosis (IPF) (Fry. *et al*., 2014).

Using stable housekeeping miRNAs as reference genes is important for accurately quantifying miRNA expression levels in respiratory tissues, as the expression of individual miRNAs can vary widely depending on the disease state and tissue type. Normalization of miRNA expression to stable reference genes can help to minimize technical variability and improve the accuracy and reliability of miRNA expression analyses in respiratory disorders (Abd-El-Fattah *et al*., 2013).

3. Materials and Method

3.1 Patients

One hundred fifty-five samples, varied between infected and healthy, were collected from Al-Mahaweel Hospital, Marjan Hospital, and Saleh Al-Mukhtar Tuberculosis Center in Babylon province / Iraq. During the period from September 2022 to February 2023. The patient's age ranged from 10 to ≥ 60 years, because those older than 60 years are available randomly. The patient is attending consultations in the hospital to get treatment. And dividers are as follows:

Total of 107 specimens of blood samples have been collected from patients with respiratory disorders. Samples distribute into some diseases, including (Asthma, TB, COPD, Corona, Bronchitis, and Sensitive bronchitis).

Exclusion criteria patient complicated with other disease such as:

1- Diabetes
2- Arthi's
3- Blood Pressure
4- Kidney disorder
5- Heart disease was excluded from study

3.2 Equipment and Apparatus

Equipment and apparatus with their company name and origins used in the presented work have been shown in the table below.

Table (3.1): The equipment and apparatus used in the study.

No	Item	Company	Country
1	Clean tube	Applied Biosystem	USA
2	Clod centerifuge	FAOR gen	Taiwan
3	Cloves	One plus	Turkey
4	Collection tube	Bio comma limited	Spain
5	Cool box	Tank	AIR
6	Cotton	Kardelen	China
7	Deep freezer (-40)	Thermo-fisher	
8	EDTA tube	Memmert	Germany
9	Eppendorf tube	Awareness technology	USA
10	Farb mini column	FaoR Gen	China
11	Gel tube	AFCO	USA
12	Incubater	DIALAB	Austria
13	Laboratory Fume hood	Gel	Germany
14	Micropipette from 0.1 -3	Dragon lab	USA
15	Micropipette from 0.5-10	Dragon lab	USA
16	Micropipette from 10 -100	Dragon lab	USA
17	Micropipette from 100-100	Dragon lab	USA
18	Nanodrop	Dragon lab	USA
19	PCR tube	Bio near	Japan
20	PCR tube rack	Watson Biolab	Turkey
21	Pipette tip (blue)	Applied biosystem	USA
22	Pipette tip (yellow)	Applied biosystem	USA
23	Precision pipttes and disposable piprttr tips		
24	RT PCR system	Qtower 3G Analytica	Germany
25	Slides	Zahrat Alrawan	China
26	Sterile material (ethand)	Aljoud	Iraq
27	Syringe	Easy med	China
28	Vortex mixer	Dragon lab	USA

3.3 Kits and their Contents

table (3.2) kits that have been used in the present study

No	Kits	Company	Country
1	Go Taqo 1-step RT-qPCR system	Promega	USA
2	Human Interleukin 13 Elisa Kit (96T)	Ela science	USA
3	Transzol up	ET 111.01	USA

3.4 chemical and Biological Materials

The chemical and biological materials that have been used in the present study are shown in the table.

Table (3-3): Chemical and biological material used in this study

No	Material	Company
1	Ethanol	Biotech
2	nuclease-free water	Promega
3	TransZol	ET 11-0.1

3.5 Molecular material

3.5.1. primers

Table (3.4) The primers employed in this investigation are displayed.

Primer types utilized in the investigation

Gen of MiRNA	sequences	Ref.
MiRNA 151	F5'-CCCCTCGAGGAGCTCACAG-3' R5'-GTGCGTOTCGTGGAGTCG-3'	**(Dong, et al,. 2016)**
MiRNA 625	F5'GGGGAGGGGGAAAGTTCTA-3' R5'GTGCGTGTCGTERAGTCG-3`	
H·kgene U6	F5'GCTTCGGCAGCACATATACTAAAAT-3' R5'-CGCTTCACGAATTTGCGTCToAT-31'	

3.6 Methods:

3.6.1 .Blood Sample Collection.

Peripheral blood (5 ml) was drowned from each patient; according to the type of study, blood samples were distributed into two different tubes, 3ml in the gel tube for the serological study and 2 ml in the EDTA tube for the first analysis complete blood count and remain for the molecular study. Serological detection was carried out using the ELISA technique, while molecular detection was carried out by using the PCR RT-PCR technique.

3.6.2 Bacteriological material.

3.6.2.1- culturing

Bacterial culture is a technique used to grow and multiply bacteria in laboratory conditions simulating natural biological conditions. Bacterial samples are collected from patients with respiratory diseases and cultured in a specific nutrient medium in the laboratory. This technique aims to identify and characterize pathogenic bacteria and understand their interactions and behavior in the respiratory tract.

Steps for bacterial culture for respiratory disease include:

In the first stage, samples of fluids or secretions from the patients' respiratory tract, such as sputum or throat fluids, were collected using laboratory instruments designated for this. After that, the sample was embalmed by placing it in a nutrient medium such as Blood Agar, love bile salt (MacConkey Agar), and NaCl 10% (Mannitol Agar), which contains the nutrients necessary for the growth of bacteria. pH and concentration of the food is adjusted to provide optimal conditions for growth of potential bacteria and then grown in a suitable culture medium. Sample was distributed over the surface of the culture medium using sterile instruments. After culturing the sample, the culture medium was placed in the incubator

providing suitable conditions for bacteria to multiply. The temperature is set at 37 degrees, humidity, and ventilation for a period ranging between 24 and 48 hours. Identification: After a period of culture, the bacterial culture is analyzed to identify and identify the species present. Techniques are used different methods such as microscopy and biochemical verification tests.

all media prepare according to manufactured company

Table (3-5) lists the chemical and biological ingredients that were utilized in the current investigation.

No	Item	Company	Country
1	Acid Fast stain	Ziehl-Neel son Stain	Jordan
2	Blood agar	Liohilchem	Italy
3	Gram stain	Ziehl-Neel son Stain	Jordan
4	MacConkey agar	Liohilchem	Italy
5	Mannitol agar	Liohilchem	Italy

3.6.2.2 Identification

Bacteria were diagnosed phenotypically according to the presence of colonies, their shape, texture, number, color, and size.

Microscopic diagnosis: using Gram stain to determine the type of bacteria whether positive or negative for this stain, and acid-fast stain.

Microscopic diagnosis refers to the use of a microscope to examine pathological specimens under a lens to determine the presence and characterization of bacteria. Microscopic diagnostics is used to check for the presence of bacteria in a sample and to determine its morphological and structural characteristics, which helps guide further examinations and tests. Steps for microscopic diagnosis include:

Dye: The treated sample is stained with a microscopic stain to improve contrast and visualize the microstructures of bacteria. Common

stains used include the Gram stain and Ziehl-Neelsen stain to stain alcoholic acid bacteria such as Mycobacterium tuberculosis Italy.

Observation under a microscope: The colored sample is placed on a glass slide and placed under a microscope for analysis. A microscope is used to observe the sample and identify bacteria present and morphological structures such as shape, size, and distribution.

Characterization and analysis: Bacteria seen are characterized and analyzed based on the characteristics visible under the microscope. The shape of the bacteria (such as spherical or rod-shaped), the assemblages (such as nodules or chains), and other characteristics associated with the potential bacterial species are determined.

Biochemical test

Gram stain: This is a rapid staining technique used to identify bacteria based on their cell wall characteristics. It provides preliminary information about the type of bacteria present and helps guide further testing.

Oxidase Test:

The oxidase test is used to determine the presence of the oxidase enzyme in bacteria. This is done by placing a small drop of the oxidase solution on a disc of paper impregnated with a special chromogenic substance. If the bacteria have the enzyme, the chromogenic material will be turned blue-black (Cortes-Borrego., 2023)

Catalase Test:

The catalase test is used to determine the presence of the enzyme catalase in bacteria. This is done by placing a drop of hydrogen peroxide solution on the cultured bacteria. If the bacteria have the enzyme, the hydrogen peroxide will decompose into water and oxygen, resulting in the formation of gas bubbles.

IMViC Tests:

IMViC is an acronym for four different tests: the indole test, the methyl red test, the Voges-Proskauer test, and the citrate test. These tests are used to identify different strains of coliform bacteria, including some types associated with diseases.

Culture and sensitivity testing:

A sample from the respiratory tract, such as sputum, throat swab, or bronchoalveolar lavage, is collected and cultured in a laboratory. The bacteria are allowed to grow in a specific medium, and their characteristics are observed. This test helps identify the specific bacteria causing the infection and determine the most effective antibiotic treatment.

3.6.3 Complete blood count or hematological test

The complete blood count (CBC) includ the total and differential count of the white blood cells (WBCs). the CBc test is automatically done by Beckmen Coulter Analyzer system.

3.6.4 Serological assay.

1 .All reagents were brought to room temperature (18-25°C) before use. The strips and reagents needed for the current experiment were removed, and the remaining strips and reagents were stored.

2 .Washing solution: 30 ml of concentrated washing solution was diluted with 720 ml of deionized water or distilled water to prepare 750 ml of washing solution.

3 .Standard working solution: Standard centrifuge at 10,000 × g for 1 minute. 1.0 ml of standard diluent and reference sample were added, left for 10 minutes and gently stirred several times. After it dissolves completely, mix it well with a pipette. This reconstitution produces a working solution of 1000 pg/mL. Serial dilutions were made as needed.

The recommended dilution gradient is as follows: 1000, 500, 250, 125, 62,500, 31,250, 15.63, 0 pg/mL. Dilution method: 7 EP tubes were taken, and 500 µL of reference standard and sample diluent were added to each tube. 500 µl of the 1000 pg/ml working solution was pipetted into the first tube and mixed to produce a 500 pg/ml working solution. Pipette 500uL of solution from the previous tube to the last tube according to this step.

4 .Detection of Biotinylated Ab Working solution: The required amount was calculated before the experiment (100 µl/well). In preparation, a little more was prepared than calculated. Centrifuge the concentrated biotinylated Ab at 800× g for 1 minute, then dilute 100× the concentrated biotinylated Ab to 1× working solution with the concentrated biotinylated Ab Diluent (concentrated biotinylated Ab Diluent = 1:99).

5. HRP Conjugate Working Solution: HRP Conjugate is HRP conjugated avidin. The required amount was calculated before the experiment (100 µl/well). In preparation, the concentrated HRP conjugate was centrifuged at 800 × g for 1 min, and then the concentrated HRP conjugate was diluted 100× to 1× working solution with conjugated HRP diluent (concentrated HRP conjugate: conjugated HRP diluent = 1:99). The working solution must be prepared immediately before use.

Perform the examination

1 .Wells were designated for diluted, blank, and sample standards. Then 100 µL of each dilution of the standard, blank, and sample were added into the appropriate wells. The plate is coated with the sealant provided in the kit. Incubate for 90 minutes at 37 °C.

3.6.4.1 .Estimation of IL-13 Serum levels

The level of cytokines (IL-13) was assessed in sera of respiratory disorder patients and controls by means. Of ELISA (enzyme-linked

immunosorbent assay) technique using ELISA kit for cytokines. The manufactured instructions of IL-13 kit consist of.

- 96 well microtiter strip
- Plastic plate covers
- IL-13 Standard.
- standard Diluent (Buffer)
- Standard Diluent Serum
- Bio tiny late Antibody diluent
- HRP Conjugated
- HRP Diluent
- Washing Buffer
- Substrate
- IL-13 Control
- stop solution
- Add 50 ml of stop solution. Determine the OD value at 450nm immediately

3.6.4.2 Principle of Assay

The microtiter plate wells have been Coated with an Antibody specific for IL-13. The IL-13 in standard and Samples were binding with antibody es, while the unbound IL-13 was removed during the washing step. The binding of the biotinylated anti-IL-13 hollows this. To perform a complex while the unbounded antibodies were removed during the subsequent washing step. The HRP Conjugate was then added to each well. Followed by adding The substrate, which resulted in the production of blue Color changed to yellow after adding the stop solution. The concentration of IL-13 was measured depending on the intensity of the colored complex

and the O.D values (450nm), which are used to plot a standard curve to calculate the concentration of IL-13 in the used samples.

3.6.4.3 Assay Procedure

The liquid was filtered from each well, and 100 µL of Biotinylated Detection Ab working solution was immediately added to each well. The plate was then covered with new sealant. Incubate for 1 hour at 37 °C .The solution was decanted from each well, and 350 µL of washing solution was added to each well. The solution was soaked for 1 minute and then the solution was pipetted or poured from each well and left to dry on clean absorbent paper. This washing step was repeated 3 times .100 µL of HRP conjugate working solution was added to each well. The plate was then covered with new sealant. And incubate for 30 minutes at 37°C .The solution was decanted from each well, then the washing process was repeated for 5 times as in step 3 .90µL of substrate reagent was added to each well. The plate was then covered with fresh sealant and incubated for 15 minutes at

37°C with the panel protected from light .50 µL of stop solution was added to each well .The optical density (OD value) of each well was determined once using a microplate reader set to 450 nm.

3.6.4.4 Calculation of results.

The concentration of IL-13 in the test samples was determined by drawing a standard linear curve in which the O.D of all the standards is plotted on the y-axis, while the IL-13 Concentration is on the X-axis. To determine the amount of IL-13. The O.D read against the IL-13 Concentration as in Figure 3.1

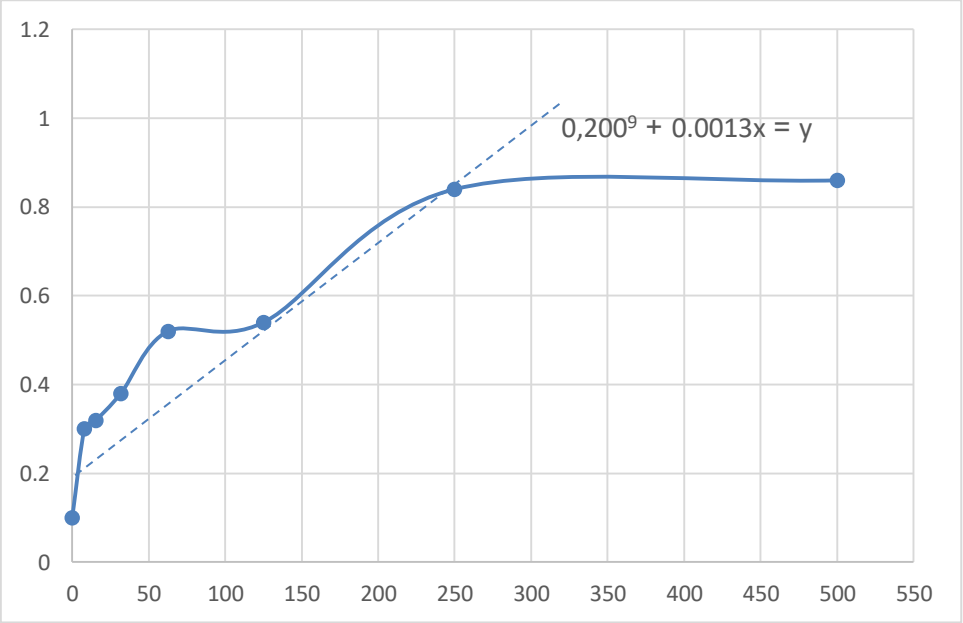


Figure 3.1 The concentration of IL-13 in the test samples

3.7 Molecular analyses:

3.7.1 Human RNA Extraction Kit:

Kit Content: TransZolup, lysis Buffer, isopropanol 751. ethanol, RNAase-Free water

3.7.1.1 Protocol: Isolation of Total RNA From Human Whole Blood:

Description:

The isolation of total RNA from human whole blood is an essential step in many molecular biology experiments, such as gene expression analysis or RNA sequencing. Here is a general protocol for isolating total RNA from human whole blood:

Materials:

-Human whole blood samples

-TRIzol reagent or other RNA extraction kit

-Chloroform

-Isopropanol

-75%ethanol

-RNase-free water

-Centrifuge

-Microcentrifuge tubes

-Pipettes and pipette tips

-RNase-free workspace and equipment

Protocol:

1 .Collect human whole blood samples using appropriate collection tubes and anticoagulants, such as EDTA or heparin. Mix the blood gently to ensure proper anticoagulation.

2 .Transfer the blood samples to sterile tubes and allow them to stand at room temperature for 10-20 minutes to allow clotting to occur.

3 .Centrifuge the tubes at a relatively high speed (e.g., 1500-2000 x g) for 10 minutes to separate the blood components. This will result in three layers: the top layer containing plasma, the middle layer containing the buffy coat (white blood cells and platelets), and the bottom layer consisting of red blood cells.

4 .Carefully remove the plasma and the buffy coat using a pipette, being cautious not to disturb the red blood cell layer.

5 .Transfer the buffy coat into a new sterile tube and add an equal volume of TRIzol reagent (or the appropriate RNA extraction reagent) to the tube. Mix thoroughly by vortexing to ensure complete lysis of the cells.

6 .Incubate the mixture at room temperature for 5 minutes to allow the complete dissociation of nucleoprotein complexes.

7 .Add an equal volume of chloroform to the tube and mix vigorously by shaking for 15-30 seconds. The chloroform will separate the mixture into three phases: the upper aqueous phase (containing RNA), the interphase (containing DNA and proteins), and the lower organic phase.

8 .Centrifuge the tube at high speed (e.g., 12,000-15,000 x g) for 15 minutes at 4°C. The centrifugation will cause phase separation, with the RNA remaining in the aqueous phase.

9 .Carefully transfer the upper aqueous phase, which contains the RNA, to a new tube, being cautious not to disturb the interphase or the organic phase.

10 .Precipitate the RNA by adding an equal volume of isopropanol to the tube. Mix gently and incubate at room temperature for 10 minutes to allow RNA precipitation.

11 .Centrifuge the tube at high speed (e.g., 12,000-15,000 x g) for 10 minutes at 4°C to pellet the RNA.

12 .Discard the supernatant without disturbing the RNA pellet. Wash the RNA pellet by adding 1 mL of 75% ethanol, and centrifuge at a lower speed (e.g., 7,500 x g) for 5 minutes at 4°C.

13 .Discard the ethanol and air-dry the RNA pellet for approximately ten to fifteen minutes, or until all traces of ethanol have evaporated.

14 .Resuspend the RNA pellet in an appropriate volume of RNase-free water. The volume will depend on the expected concentration and downstream applications.

15. Store the RNA at -80°C or proceed with downstream applications such as reverse transcription or RNA sequencing.

3.7.1.2 Go Tad 1-step RT-qPCR system kit.

Product components.

Table (3.6) Includes Product components:

substance	Quantity
Gotaq qPCR Master Mix,	5 x 1 ml, 2X
Go scriptTM RT Mix for 1-step RT-qPCR	225 ml
CXR Reference Dye	200 ml, 30 mM
MgCl2,	750 ml, 25 mM
nuclease-free water twice.	13 ml

3.7.1.3 General Considerations of Instrument

Considerations for the instrument, RNA template, BRyt Green® dye, CXR Reference Dye, and qPCR primers

3.7.1.4 .Gotaq 1-step RT-qPCR protocol

A-CXR Reference was added Dye to the Gotaq qPCR Master mix. Some real-time PCR instruments need greater concentrations of CXR reference dye; add CXR reference dye to reach a high dye concentration (500nm) for high reference dye devices. the following

1 .Gotaq R qPCR Master Mix was first thawed.

2 .For mixing, the Gotaq R qPCR Master mix was cycled for three to five seconds.

3 .0.33 mL was added for every 20 mL of reactions when a high reference dye was used to obtain a final concentration of 500 nM.

4. To mix, the vortex was used for 3 to 5 seconds.

3.7.1.5 The GoTaq 1-step - qPCR Reaction mix is put together.

The final response volume for the protocol is 20 mL.

It is possible to adjust the sizes shown here for larger or smaller interaction sizes.

1 .Exonuclease-free water and GoTaq R qPCR Master Mix were dissolved.

2 .The Go TaqR qPCR master mix was cycled for 3–5 seconds. Low speed vortex prevents aeration for perfect collection.

3 .The number of reactions to be set up was determined, and one or two additional reactions were added to this number to compensate for any negative control reactions.

4 .The Gotaq qPCR master mix, excluding the RNA template, was fused to get the reaction ready for mixing. Go to script for PCR primers, nuclease-free water, and RT mix.

5 .The reaction mixture was properly diluted and applied to each PCR tube or well of an optical grade PCR plate.

6 .The RNA template was added to the appropriate wells of the reaction plate (or water for the control reaction without template.

7. The photovoltaic panels or tubes are closed. plus centrifuge briefly to collect the contents of the bottom and bottom wells; Protection against prolonged exposure to light or high temperatures. Thermal cyclinization is now possible with samples.

Table (3-7). Gotaq RT-qPCR Reaction Mix, one step.

Component	Volume per 20ml Reaction	Volume per 50ml Reaction	Final concentration in reaction
Go Taq® qPCR Muster Mix, 2x	10ml	25ml	1x
Forward primer, 10x	2ml	5ml	50-300nm
Revers primer, 10x	0.4ml	10ml	50-300nm
Gu script ^{Tm} RT Mix hor 1- step RT-qPCR, 50X or Nudease-free water for Minus-RT Control	4ml	10ml	1x
RNA template (500fg-loung nq) or Nuclease-free water For No-Template Controll	4ml	10ml	variable
optional: Mgcl2,25m M*	-M1	-M1	$\geq 2mM$
optional: CXR reference Dye, 30µM	-M1	-M1	$\geq 33mM$
Nuclease-Free water	To 20 ml	To 50µl	-

3.7.1.6 Energy Cycles

The cycling parameters below are provided as a general guideline and can be changed for the best results.

Table (3-8) General thermo cycler program of qPCR

step	cycles	Temperature	Time
Reverse transcription	1	≥ 37℃	15 min
Inactivation of reverse transcriptase and activation of Gotoy/RADNA polymerase	1	95℃	10min
Denaturation		95℃	10se
Annealing and data Collection	40	60℃	30se
Extension		72℃	30se

Table (3-9). Summarized Rinorophones, Dyes, Emission and excitation parameters used for multiplex RTPC R Modules

Pos.	Quencher	Excitation	Detection	Dye	Gain	Measurement	Pass. Ref
1	Blue	470	520	Fam	5	Yes	No
2	Green	515	545	JoE	5	No	No
3	Orange	565	605	Rox	5	Yea	No
4	Red	630	670	Cy5	5	No	No

3.7.1.7 .Calculating Gene Expression (Fold change)

The two methods of qPCR data analysis are absolute and relative quantification. Relative quantification determines changes in gene expression relative to a reference gene, whereas absolute quantification defines the input gene account based on a standard curve. When constructing a standard curve, a sample was used that was accomplished by errors caused by standard dilutions. is also avoidable.

More intriguing than precise DNA/RNA molecule counts are groupings. As a result, relative quantification is carried out absurdly.

Gene expression or gene hold or Value Calculated. RQC Relative quantification،

RQ = 2-(ΔΔCT)

Gene holds, or RQ is Calculated freshly by collecting CT (CT-cycle threshold or CQ-cycle quantification) average value from a real-time PCR device for each Triplicate Sample, then calculating ACT value for both treated and untreated samples as follows:

ΔCT=CT (gene of interest) - CT (reference gene) To calculate ΔΔCT value which was found as hollow:

ΔΔCT=ΔCTC Treated Sample) - ΔCT (untreated sample control)

After Calculating ΔΔCT for all samples then, take the Final equation to Calculate Gene expression or RQ is as follows:

Fold change = 2-(ΔΔCT).

3.8 Statistical analysis

Data was analyzed using SPSS (version 26, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard Error), Statistical analysis was carried out using chi-square, the value of $p \leq 0.05$ and 0.01 was considered to be statistically significant. As well as differences were compared by One-way ANOVA at $p \leq 0.05$ using Duncan's Multiple Range test. The relationship between studied parameters was determined by Pearson's correlation coefficient (r). Receiver-operating-characteristic (ROC) curves were analyzed to evaluate the performance for disease detection.

4.Results and Discussion

4.1. Demographic Distribution

4.1.1. Gender Distribution:

One hundred fifty-five samples were collected from patients and control males and females. The distribution of the sample was 48 controls 30.52% of the total sample, of which 31 were male samples 64.58% of the total control, and 17 were female samples 35.42% of the total control. And 107 were patients representing 69.48% of the total sample, of whom 42 were male representing 39.25% of the total patient sample, and 65 were females, representing 60.75% of the total patient sample. The distribution of the sample was 73 males, representing 47.7% of the total sample, and 82 females 52.93% of the total sample. All of this is shown in Table 4.1.

Table (4.1): Sample distribution according to gender.

Gender	Control		Patient		P-value
	No.	%	No.	%	
Male	31	64.58	42	**39.25**	**0.641****
Female	17	35.42	65	60.75	**0.641****
Total	48	100	107	100	

** P-valu is significant at the 0.01.

The study shows that the prevelance of respiratory diseases is prophetically higher in females than in males, and with regard to the higher incidence of respiratory diseases between males and females, the sex distribution can vary based on the type of disease and other factors. In some cases, there may be a difference in the sex ratio, while in others there may not be differences. For some respiratory diseases, such as tuberculosis

and inflammation of the lower airways (such as bronchitis and pneumonia), males are more susceptible (Hu *et al*., 2023). In some cases, this can be due to factors such as occupational exposure to pollution or unhealthy health behaviors (such as smoking) (Abbas *et al*., 2023). On the other hand, females may be more susceptible to certain conditions such as asthma and certain respiratory allergies (Chapman 2004). However, it should be noted that the findings can it varies between studies and different geographic regions and may be affected by other factors such as age, family history, and environment. This is consistent with the study (Adeniyi *et al*., 2017) and (Lv, *et al*, 2023).

4.1.2. Age group distribution:

patient and healthy distributed with many age group. The total numbers of patients with respiratory diseases were 107 and healthy control was 48. The higher number of patients group > 50 years old (40 patient) 37.38% and The lower number of patient appeared in age group 41 to 50 years old (11patient) also the Highter number of healthy individuals appeared in age group 21-31 years old (14 patient, 29.17%) and the lower number appeared in age group >50 years old (4 patient, 8.33%). Shown in table 4.2

Table (4.2): Sample distribution according to age groups.

Age (year)	Control		Patient		P- value
	No.	%	No.	%	
10-20	11	22.92	17	15.89	.508**
21-30	14	29.17	23	21.5	.446**
31-40	8	16.67	16	14.95	.632**
41-50	11	22.92	11	10.28	.462*
>50	4	8.33	40	37.38	.804**
Total	**48**	**100**	**107**	**100**	

**Correlation is significant at P-value = 0.01.

*Correlation is significant at P-value = 0.05.

From Table 4.2 and noted that the highest incidence of respiratory diseases is in the age groups over 50 years old, which was similar to the results of (Savic *et al.*, 2023). Weak immune system: In older people can have an immune system Less effective in fighting infections and diseases. This weakness can lead to their susceptibility to various diseases including respiratory diseases (Azarbakhsh *et al.*, 2023). Another reason for accumulating exposure to harmful factors: Older people may be subject to repeated exposure to environmental pollutants and various harmful factors over the years. For example, they may be more likely to smoke or have a long history of exposure to air pollutants in their surroundings. This accumulation may increase the risk of respiratory disease (Chenchula *et al.*, 2023). As for the second higher category in terms of infection with respiratory diseases, it is for ages between 21 to 30 years, which the attributed to the reason that the majority of people with respiratory diseases in the research samples are Covid-19 diseases, and the reason for this is that young people are the most prevalent in society, which exposes

them to the most possibility for infection, where people infected with Covid 19 disease were 51% of the total research sample, at Table 4.4. The result were indicated by (Karami, *et al*, 2023) in their study. And then comes the age group 10 to 20 years, and the reason for this is attributed to each of (Franjic, 2019; Jartti, *et al*, 2019) It is likely that the severity of respiratory diseases depends on the age of the child, the type of virus or their combination, the presence of atopy, and other factors Environmental (climate, secondhand smoke), immune interaction, and genetic predisposition. However, it should be noted that these percentages may vary between countries and regions, and may be affected by other factors such as environment and lifestyle. Which came close to the study (Lewnard, *et al*, 2023).

4.1.3 Smokers distribution

The research sample was distributed in terms of smoking or not, where the number of smokers was 15 smokers, of which 4, 8.33% of the total number of controls. And 11 patients, representing 73.33% of the total number of smokers, and 10.28% of the total number of patients. As for non-smokers, they numbered 140 samples, representing 90.32% of the total sample, of whom 44 were controls, representing 31.42% of the total number of non-smokers, and 91.67% of the total number of controls. The number of non-smokers was 96 samples, representing 68.58% of the total number of non-smokers and 89.72% of the total number of patients. All of this is shown in Table 4.3.

Table (4.3): Sample distribution according to smoking.

Smoking	Control		Patient	
	No.	%	No.	%
Yes	4	8.33	11	10.28
No	44	91.67	96	89.72
Total	48	100	107	100

The current study showed that the proportion of non-smokers is higher than it is in smokers, and this was contrary to all studies, as there were 60% of people with respiratory diseases who did not smoke in the research sample (Yang *et al*., 2022). Some possible explanations exposure to environmental pollution Non-smokers may be exposed to air pollution with harmful substances such as toxic gases, particulates and industrial exhausts. This contamination may cause respiratory diseases (Javorac *et al*., 2023). Allergies and sensitivities Non-smokers may have an allergy or sensitivity to certain substances in the environment such as pollen, dust, or fungus. These sensitivities can irritate the respiratory system and produce symptoms similar to respiratory diseases. Exposure to occupational factors non-smokers may be exposed to harmful factors in the workplace such as dust or chemicals. These factors may cause them to develop respiratory diseases (Valbert, *et al*, 2022). Exposure to genetic factors Some non-smokers may have genetic factors that make them more susceptible to respiratory diseases. They may have a family history of respiratory diseases or variations in certain genes that make them more susceptible to these diseases. The last reason is exposure to passive smoking, i.e. respiratory disease for smokers, which was indicated by (Detorakis, *et al*, 2023).

4.1.4 Type of disease distribution

In terms of disease distribution among patient samples, which totaled 113 samples, or 69.48% of the total number of samples, 55 of them were infected with Covid-19, or 48.67% of the total number of patients. As for the patients with asthma, they numbered 28 patients، or 24.77% of the total number of patients. There were 5 patients with Tuberculosis, 4.42% of the total number of patients. There were two patients with COPD or 7.07% of the total number of patients. patients with Bronchiolitis were 11, or 9.73% of the total number of patients. Those with sensitive bronchitis were 6, or 5.30% of the total number of patients. All of this is shown in Table 4.4.

Table (4.4): Patient samples distribution according to the type of disease.

Type of disease	No.	%	P -value
Covid-19	53	49.53	**≤0.0001****
Asthma	23	21.49	
Tuberculosis	6	5.60	
COPD	8	7.47	
Bronchoititis	11	10.28	
Sensitive bronchitis	6	5.60	
Total	**107**	**100**	

**** significant difference at p≤0.05**

The results showed that the disease with the highest incidence among respiratory diseases is Covid 19 disease. Then asthma, and then sensitive bronchitis, and the rest of the diseases came in close proportions. The higher incidence of coronavirus (COVID-19) compared to other respiratory diseases can be explained by several factors of the spread of infection (Verity ., 2020). Coronavirus spread globally very quickly and affected a large number of populations around the world. The virus is easily

transmitted by respiratory tar through droplets produced when coughing, sneezing, or even talking. This greatly increases the chances of contracting the virus compared to other respiratory diseases that may be less contagious (Tsampasian *et al.*, 2023). Corona virus has several characteristics that increase its spread and transmission quickly. A person infected with the virus can be contagious even before symptoms appear, which means that many people can transmit the virus without knowing it. This promotes the spread of the virus. Preventive measures despite efforts to spread health awareness and implement preventive measures, such as social distancing, wearing masks and washing hands, the Corona virus is still spreading widely in some communities. This may be due to the challenges of properly applying or sometimes bypassing safeguards. Corona virus may cause severe symptoms in the respiratory system, including severe pneumonia, which increases its risks and impact on individuals. People with severe symptoms may require intensive care or intensive medical intervention, which will increase awareness and investigation of infection with the virus (Mohamed *et la.*, 2023). As for asthma, which came second in the classification in terms of infection rates, the outcome of this study was similar to the study of (Desalu *et al.*, 2009), in which asthma came second as well, and the reason for this is due to genetic factors and environmental pollution, and this is what Desalu also mentioned in his study as well. As for the researcher's opinion, in the post-Corona era, which is the first disease in infection rates due to the speed of infection.

4.2 Hematological Test:

4.2.1 White Blood Count test and deferential White Blood Count.

The White Blood Cell count is a common blood test that measures the total number of white blood cells in a sample of blood. White blood cells are an essential part of the immune system and play a crucial role in defending the body against infections. The normal range for WBC count can vary slightly depending on the laboratory, but it typically falls within the range of 4,000 to 11,000 white blood cells per microliter of blood. An abnormal WBC count may indicate various conditions, such as infections, inflammatory disorders, leukemia, or immune system disorders. It is essential to interpret the results in the context of the individual's overall health and other clinical findings (Thungathurthi, and Orsu, 2022).

The differential WBC count provides a breakdown of the different types of white blood cells present in the blood. There are several types of white blood cells, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The differential count expresses the percentage of each type of white blood cell in the total WBC count.

Table (4.5) White Blood Count test and deferential White Blood Count.

Biomarkers / Groups	WBCs	Neutrophils	Lymphocytes	Basophiles	Eosinophytes	Monocyte
	Mean±S.E					
Control	6.53±1.19	30.74±1.01	23.5±12.1	0.114 ± 0.035	2.9 ± 1.05	0.30±0.90
Covid-19	7.87±0.6 [a]	58.49±4.5 [a]	25.43±1.8 [ab]	1.52±0.3 [c]	6.2±1.2[c]	0.56±0.02 [b]
Asthma	13.23±1.1 [b]	60.90±3.7 [a]	23.32±2.2 [a]	1.33±0.2 [b]	5.5±0.9[b]	0.42±0.01 [a]
Tuberculosis	16.22±2.2 [c]	75.16±8.9 [c]	32.44±2.6 [c]	0.82±0.1 [a]	5.1±0.7[b]	0.39±0.01 [a]
COPD	8.95±1.7 [a]	69.95±5.5 [b]	28.20±1.2 [b]	1.61±0.4 [c]	7.3±1.8 [d]	0.59±0.03 [b]
Bronchiolitis	7.78±1.3 [a]	60.66±7.2 [a]	26.11±4.6 [b]	1.22±0.3 [b]	4.9±1.1 [ab]	0.70±0.03 [c]
Sensitive bronchitis	12.95±1.9 [b]	60.05±4.3 [a]	23.80±5.2 [a]	0.96±0.2 [a]	4.2±0.6 [a]	0.66±0.01 [bc]

-Different letters refer to significant difference at $p \leq 0.05$.

From Table 4.5, the values of WBCs for a disease in terms of WBCs are the highest in tuberculosis with a mean of 16.22 ± 2.2 and this value was close to the study (Thungathurthi and Orsu, 2022). Then comes asthma, with a mean of 13.23 ± 1.1, and the value was similar to the study of (Bedolla-Barajas *et al*., 2022), followed by sensitive bronchitis, with a mean of 12.95 ± 1.9, and this value was similar to the study of (Aronen *et al*., 2019), and all results were significant. p value < 0.05.

From Table 4.5, the values of the WBCs test for a disease in terms of Neutrophils are the highest in tuberculosis with a mean of 75.16 ± 8.9, and this value was close to the study (Thungathurthi, and Orsu, 2022). Then comes the COPD with a mean of 69.95 ± 5.5, and the value was close to the study of (Tuleta *et al*., 2017), than the rest of the diseases in similar proportions and all results were significant under p value < 0.05.

From present result we note that the values of complete blood count test for lymphocytes are the highest in tuberculosis with a mean of 32.44 ± 2.6, and this value was close to the study (Thungathurthi, and Orsu, 2022). Then the rest of the diseases in close proportions. than the rest of the diseases in similar proportions. and all results were significant under p value < 0.05.

The CBC values for Basophiles are the highest in COPD with a mean of 1.61 ± 0.4 and this value was close to the study (Tuleta *et al*., 2017). Then, Covid-19, with a median of 1.52 ± 0.3, and this value was close to the study (Bayraktar *et al*., 2023). WBCs values for Eosinophytes are highest in COPD with a median of 7.3 ± 1.8 and this value was close to the study (Tuleta *et al*., 2017). Then, Covid-19, with a median of 6.2 ± 1.2, and this value was close to the study (Bayraktar *et al*., 2023) Then the rest of

the diseases in close proportions. than the rest of the diseases in similar proportions. and all results were significant under p value < 0.05.

The values of complete blood count test in terms of monocyte are highest in Bronchiolitis disease with a mean of 0.70 ± 0.03 and this value was close to the study (Shalaby *et al*., 2023). Then, Sensitive bronchitis with a median of 0.66 ± 0.01, and this value was close to the study (Aronen *et al*., 2019).

4.2.2 Identification of Respiratory Disorder Bacterial Isolates

The results in Table 4.6 showed that for sixteen asthma specimens, 5 of which were *Streptococcus pneumoniae*, 6 of which represented *Streptococcus pyogenic*, 3 of which were *Staphylococcus aureus*, and 2 of which were Klebsiella. The appearance of these types of bacteria indicates a possible association between these bacterial types and asthma. These bacteria are known to cause respiratory infections and may play a role in exacerbating asthma symptoms.

Table (4.6) Identification of Respiratory Disorder Bacterial Isolates

Type of disease	No. of specimens	*Streptococcus pneumoniae*	*Streptococcus pyogen*	*Staphylococcus aureus*	*Tuberculosis mycobacterium*	*Klebsiella pneumonia*	*Moraxella catarrhalis*
Asthma	16	5	6	3	-	2	-
TB	6	-	-	-	6	-	-
Covid-19	11	9	2	-	-	-	-
Total	33	14	8	3	6	2	-

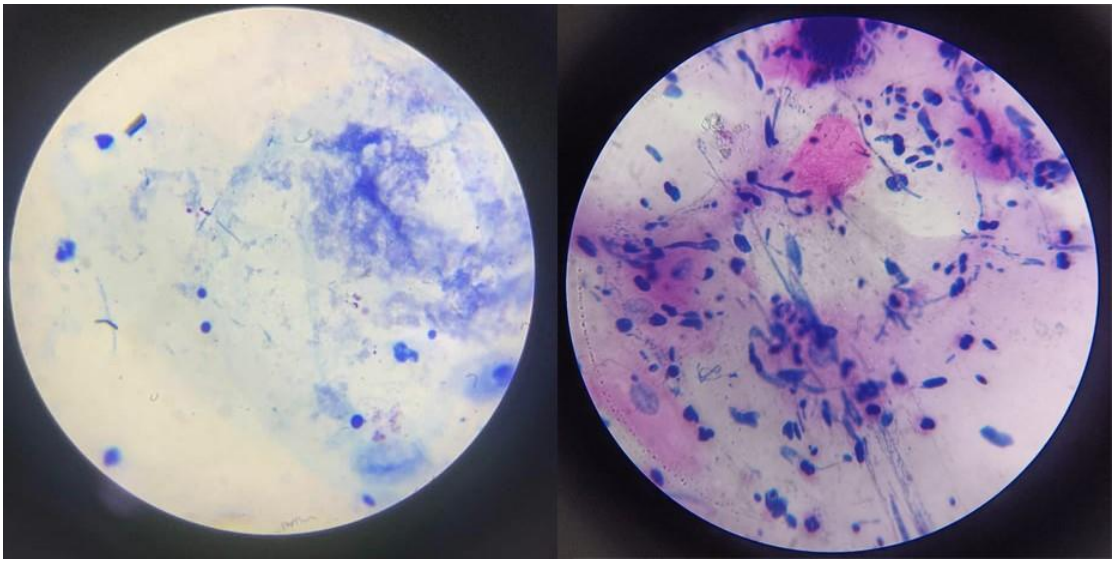


Figure (4.1) bacterial Isolates of *Tuberculosis* Under the microscope

The results shown in Table 4.6 and Figure 4.1 showed that for six specimens of tuberculosis, all isolates showed tuberculosis bacteria. Tuberculosis is a bacterial infection caused by *Mycobacterium tuberculosis*, and its presence in the samples indicates the presence of an active TB infection. It is clear that there are no other bacterial species in these samples, as tuberculosis is mainly caused by a specific pathogen.

The presence of *Streptococcus pneumoniae* and *Streptococcus pyogenic* in 11 (COVID-19) specimens suggests a possible co-infection or colonization. It is an important to note that respiratory infections, including bacterial infections, can occur in individuals with COVID-19, particularly when the immune system is compromised. The absence of *Staphylococcus aureus*, *tuberculosis*, and *Klebsiella* in these specimens indicates that these specific bacteria were not detected in the samples analyzed.

Overall, these results provide preliminary insights into the bacterial species associated with respiratory disorders such as asthma, tuberculosis, and COVID-19. However, it is important to interpret these findings in the context of larger studies, as the numbers provided represent limited sample

size. Further research and investigation are necessary to establish the significance of these bacterial species in the pathogenesis, severity, and treatment response of these respiratory conditions. Additionally, factors such as patient demographics, geographical location, and comorbidities may influence the presence and impact of bacterial species in these diseases.

4.3 Immunological Test:

4.3.1 IL 13 value for Respiratory disorder patients and control:

Based on Table 4.7, it can be observed that IL-13 levels vary among different respiratory disorders. Covid-19 and tuberculosis show increased IL-13 levels (14.5) compared to the control group, while asthma, COPD, and bronchitis exhibit similar levels to the control group. Sensitive bronchitis, on the other hand, demonstrates significantly elevated IL-13 levels. These differences suggest variations in the immune and inflammatory responses associated with different respiratory disorders. In conditions such as asthma and allergic inflammation, elevated levels of IL-13 has been observed. It is associated with airway hyperresponsiveness, mucus production, and the recruitment and activation of immune cells involved in allergic reactions (Engel, *et al*. 2023). IL-13 is also implicated in the pathogenesis of other respiratory disorders, including chronic obstructive pulmonary disease (COPD) and certain types of bronchitis (Higham *et al*., 2023).

In a control group without any respiratory disorders, the levels of IL-13 would typically be lower compared to individuals with respiratory disorders. The specific mean value would depend on the study, patient population and experimental methods used (Rebuli *et al*,. 2023).

Table (4.7) The mean of IL 13 value for Respiratory disorder patients and control

Biomarker Groups	IL13 Mean±S.E pgm
Control	9.3±1.4 [a]
Covid-19	12.3±1.8 [b]
Asthma	9.0±1.7 [a]
Tuberculosis	14.5±0.8 [b]
COPD	10.0±2.1 [a]
Bronchoititis	9.13±1.1 [a]
Sensitive bronchitis	19.0±3.6 [c]

-Different letters refer to significant difference at p≤0.05.

Diagnosing the level of cytokines in the blood can help identify and diagnose many health conditions, such as inflammatory diseases and diseases resulting from a deficiency of the immune system. This information is used to determine the appropriate treatment for the disease (Nayci and Dogan, 2023).

conducted a study in which they looked at 473 asthma sufferers and 1,892 healthy controls to see whether or not any of the almost 300,000 genetic single nucleotide variations were associated with asthma.(Prieto *et al*.,2000)

4.3.2 The ΔCT value of MiRNA 151 in Respiratory disorder patients and control

Cycle Threshold (CT) is the number of cycles required for the amplification signal to pass the background threshold. In the PCR (Polymerase Chain Reaction) technique, the CT value is used to determine the amount of target DNA in a given sample. ΔCT (Delta CT) expresses the difference in the CT value between a target sample and a reference sample. ΔCT is used to estimate the relative changes in gene or biosynthetic expression between the different groups in the study.

The role of MicroRNAs is ascertained to be vital in virus–host interactions, pathogenesis, and host resistance via regulation of post-transcriptional or translational modification. The gene expression levels of two miRNAs, MiRNA151 and MiRNA625, were analyzed in this study of Respiratory disorder patients and control.

Table (4.8): MiR151 ΔCT value

Biomarkers Groups	CT 151	CT U6	Δct	folding
	Mean±S.E			
Control	34.46±0.8	15.96±1.5	18.50±0.5	1.64±0.5
Covid-19	31.71±1.6	17.86±3.3	13.85±0.2	26.42±4.2
Asthma	31.33±0.7	15.07±1.8	16.26±1.2	12.85±2.9
Tuberculosis	35.44±1.8	18.14±1.6	17.30±1.9	9.44±1.5
COPD	30.50±1.1	16.96±2.4	13.54±0.4	35.32±1.1
Bronchoititis	30.01±0.9	15.40±0.4	14.61±0.7	24.50±1.2
Sensitive bronchitis	32.06±0.1	14.85±1.3	17.20±0.6	4.06±2.4

Correlation is significant at P-Value 0.01 level (2-tailed).

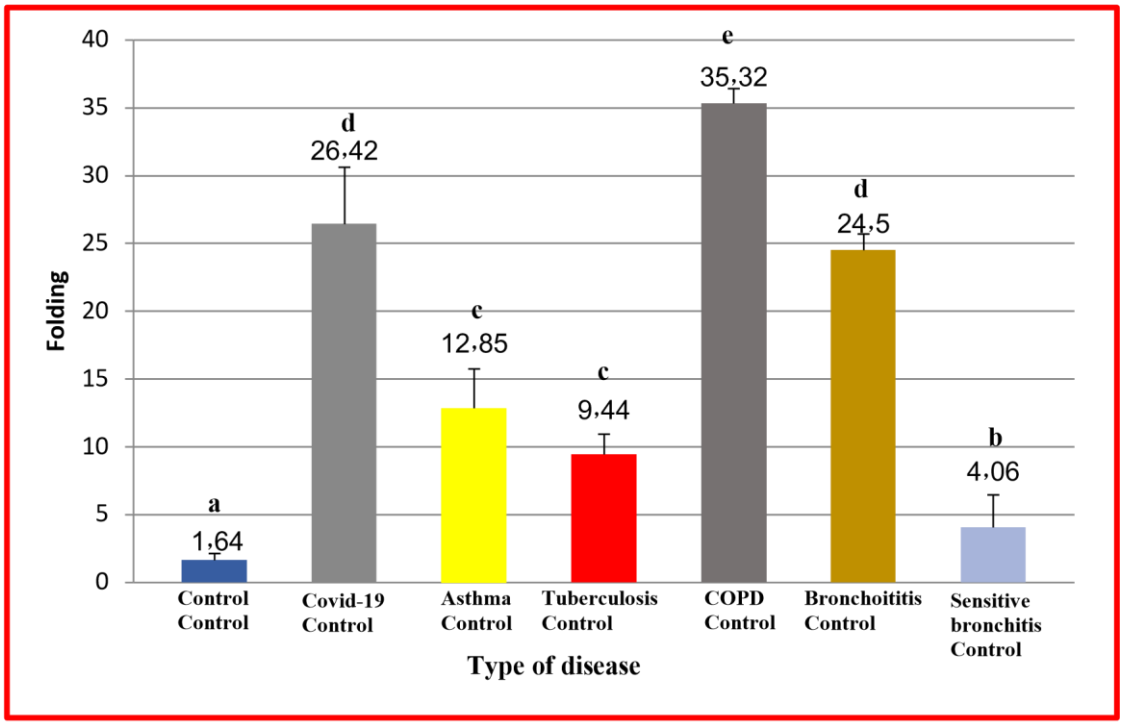


Figure (4.2) MiR151 genetic change

Figure 4.2 shows that the highest MiR151 genetic change was in COPD, with a mean folding of 35.32, followed by Covid-19, with a mean folding of 26.42, then bronchitis, with a mean folding of 24.5, then asthma, with a mean folding of 2.85, followed by tuberculosis, with a mean folding of 9.44, and finally, sensitive bronchitis, with a mean folding of 4.06. and show abnormalities or changes in Respiratory disorder patients in most of the studies and the relationship between the gradual accumulation of molecular alterations and Respiratory disorder patients has been established by the earlier studies. A homeostasis process imbalance occurs in chronic inflammatory disorders as a result of epigenetic alterations brought on by altered miRNA expression. The overexpression of pro-inflammatory cytokines and the inhibition of anti-inflammatory molecules are the results of these alterations. Numerous studies have emphasized the part miRNAs play in the development of chronic inflammatory disorders (Allegra, *et al*,. 2012; Tasena, *et al*. 2018; Liang, *et al*. 2023).

4.3.3 The ΔCT value of MiRNA 625 in Respiratory disorder patients and control:

Based on the provided values for the control group (CT, ΔCT, and folding) and the values for COVID-19 disease (CT, ΔCT, and folding) related to miRNA 625 shown in Table 4.9.

Table (4.9): ΔCT value of MiRNA625 for respiratory system patients.

Biomarkers Groups	CT625	CT U6	ΔCT	folding
	Mean±S.E			
Control	24.97±1.0	18.57±3.6	6.40±0.3	1.20±0.2
Covid-19	35.94±0.2	31.23±0.8	4.70±0.2	3.36±0.5
Asthma	31.96±0.2	28.4520±0.5	3.51±0.2	7.66±0.9
Tuberculosis	28.36±3.1	25.11±0.06	3.25±0.5	9.15±1.1
COPD	39.25±2.2	33.07±2.3	6.17±1.1	2.04±0.6
Bronchoititis	37.43±0.8	33.13±1.7	4.30±0.4	4.56±0.7
Sensitive bronchitis	38.36±0.4	32.70±1.07	5.66±0.6	2.03±0.4

Correlation is significant at P-Value 0.05 level (2-tailed).

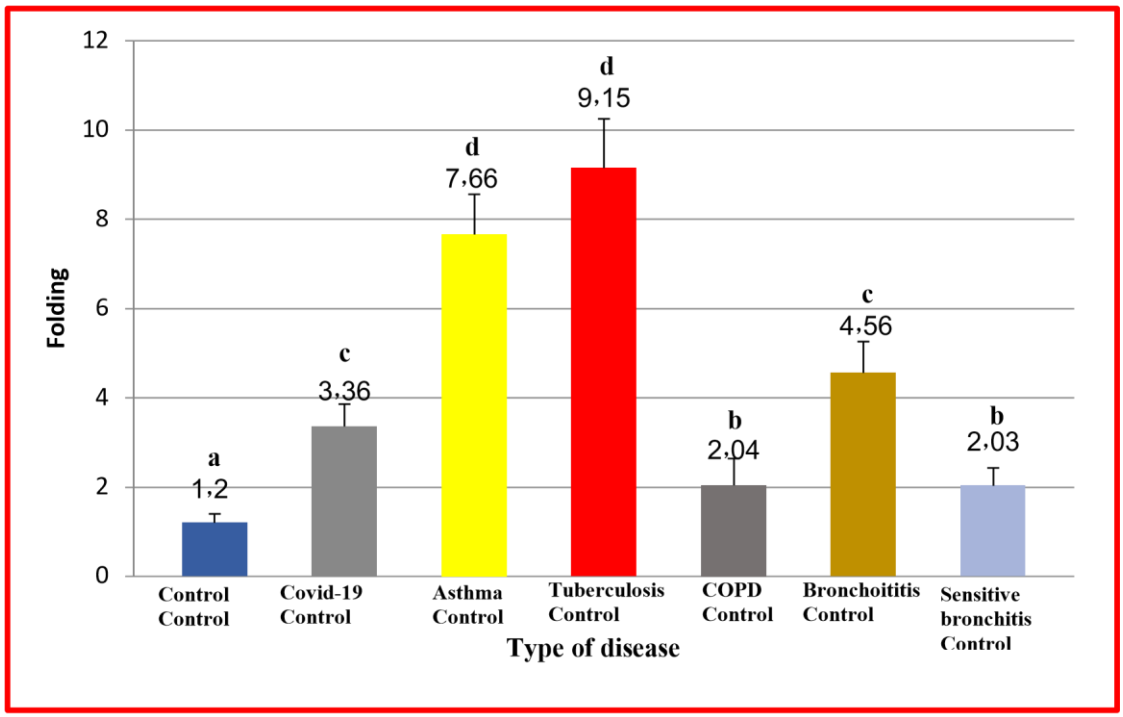


Figure (4.3) MiR625 genetic change

Figure 4.3 shows that The highest gene expression was in TB, with a mean folding of 9.15, followed by asthma, with a mean folding of 7.66, then bronchitis, with a mean folding of 4.56, then Covid-19, with a mean folding of 3.36, followed by COPD, with a mean folding of 2.04, and finally, sensitive bronchitis, with a mean folding of 2.03. The relationship between miRNA-625 and respiratory diseases has been a subject of research and investigation. Several studies have explored the expression levels and potential role of miRNA-625 in various respiratory disorders, including asthma, tuberculosis, COPD, bronchitis, and COVID-19. Here's a summary of the current understanding of miRNA-625 in relation to these respiratory diseases: Asthma***:*** Studies have shown that miRNA-625 may play a role in the pathogenesis of asthma. Aberrant expression of miRNA625 has been observed in the airways of asthmatic patients. It has been suggested that miRNA-625 may be involved in regulating the

immune response and inflammatory processes in asthma (Zhu, *et al*. 2023). Tuberculosis: MiRNA625 has been identified as a potential biomarker for tuberculosis. Differential expression of miRNA-625 has been detected in the blood, sputum, and lung tissues of tuberculosis patients. It has been proposed that miRNA-625 may have diagnostic and prognostic value in tuberculosis. COPD: MiRNA-625 has been implicated in the development and progression of chronic obstructive pulmonary disease (COPD). Altered expression of miRNA-625 has been observed in COPD patients, and it may be involved in modulating inflammation, oxidative stress, and tissue remodeling in COPD. Bronchitis: The role of miRNA-625 in bronchitis is less well-studied compared to other respiratory diseases. However, some research suggests that miRNA-625 may be dysregulated in bronchitis patients, potentially contributing to airway inflammation and tissue damage. COVID-19: Emerging evidence indicates that miRNA-625 may be associated with COVID-19. Changes in the expression of miRNA-625 have been detected in COVID-19 patients, and it has been suggested to be involved in regulating immune responses and viral replication in the context of SARS-CoV-2 infection. miR-625-5p was found to be significantly down-regulated in children with dust mite-induced asthma compared to controls, whereas the transcript levels of Cbl proto-oncogene were. These miRs may play a role in the regulation of the immune response and inflammatory cytokine pathways (Dong *et al*., 2016).

4.4 Receiver Operating Characteristic (ROC) curve test.

Table (4.10): Best cut off, sensitivity and specificity for prediction of the Some respiratory diseases activity by parameters.

Parameter		Sensitivity	Specificity	AUC	Cut off	95% confidence	ROC
Sensitive bronchitis	MiR151	0.800	0.600	0.680	1.424	0.402-0.958	**0.270**
	MiR625	0.800	0.800	0.720	1.753	0.376-1.000	**0.178**
Asthma	MiR151	0.600	0.900	0.720	3.842	0.393-1.000	**0.178**
	MiR625	1.000	0.900	1.000	2.458	1.000-1.000	**0.002****
Bronch oititis	MiR151	1.00	0.900	1.000	3.842	1.000-1.000	**0.002****
	MiR625	0.800	0.900	0.960	2.458	0.863-1.000	**0.005****
COPD	MiR151	1.000	0.900	1.000	3.842	1.000-1.000	**0.002****
	MiR625	0.800	0.800	0.760	1.583	0.414-1.000	**0.111**
covid-9	MiR151	1.000	0.900	1.000	3.842	1.000-1.000	**0.002****
	MiR625	1.000	0.900	0.980	2.381	0.919-1.000	**0.003****
TB	MiR151	0.800	0.700	0.740	2.037	0.392-1.000	**0.142**
	MiR625	1.000	0.900	1.000	2.458	1.000-1.000	**0.002****

** Significant difference refer to P≤0.005

A p-value of 0.002** for the ROC test of MiR625 for asthma indicates that the observed differences in performance are statistically significant. These values indicate that the parameter "MiR625" has perfect sensitivity. In Bronchitis The p-value of 0.002** suggests that the observed differences in performance are statistically significant. These results suggest that the "MiR151" parameter is a highly accurate predictor of disease activity in Bronchitis, with perfect sensitivity, high specificity, and excellent discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cut-off threshold. . The p-value of 0.005** suggests that the observed differences in

performance are statistically significant. these results suggest that the "MiR625" parameter is a highly accurate predictor of disease activity in Bronchitis, with a good sensitivity, high specificity, and excellent discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cut-off threshold.

In COPD The p-value of 0.002** suggests that the observed differences in performance are statistically significant. These results suggest that the "MiR151" parameter is a highly accurate predictor of COPD disease activity, with perfect sensitivity, high specificity, and excellent discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cut-off threshold.

In Covid-19 The p-value of 0.002** suggests that the observed differences in performance are statistically significant. These results suggest that the "MiR151" parameter is a highly accurate predictor of COVID-19 disease activity, with perfect sensitivity, high specificity, and excellent discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cut-off threshold. The p-value of 0.003** suggests that the observed differences in performance are statistically significant. These results suggest that the "MiR625" parameter is a highly accurate predictor of COVID-19 disease activity, with perfect sensitivity, high specificity, and very good discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cutoff threshold. In TB The p-value of 0.002** suggests that the observed differences in performance are statistically significant. These results suggest that the "MiR625" parameter is a highly accurate predictor of TB disease activity, with perfect sensitivity, high specificity, and

excellent discriminatory power. It can be considered as a strong indicator of disease presence when the value exceeds the cut-off threshold.

4.5 correlation coefficient IL13 and MiRNA151 and MiRNA625

The outcome demonstrates a direct link between respiratory system disorders and MiRNA151, MiRNA 625, and IL-13 at a P. Value of (0.01), respectively. According to this finding, elevated IL-13 levels will result in elevated expression of the MiRNA151 and MiRNA625 genes. Table (4.11) contains a list of these outcomes.

Table (4.11): Pearson correlation coefficient among biomarkers

			MiR151	MiR625
Sensitive bronchitis	IL13	r	-.603	-.793
		Sig.	.281	.110
	MiR151	r	1	.513
		Sig.		.377
Asthma	IL13	r	.850	-.767
		Sig.	.068	.130
	MiR151	r	1	-.966**
		Sig.		.008
Bronchiolitis	IL13	r	-.191	.691
		Sig.	.758	.196
	MiR151	r	1	.145
		Sig.		.815
COPD	IL13	r	-.508	-.105
		Sig.	.383	.867
	MiR151	r	1	-.490
		Sig.		.403
Covid-19	IL13	r	-.131	.374
		Sig.	.834	.535
	MiR151	r	1	.796
		Sig.		.107
TB	IL13	r	.004	-.656
		Sig.	.995	.229
	MiR151	r	1	.081
		Sig.		.896

**. Correlation is significant at the 0.01 level (2-tailed).

The immune system plays a crucial role in defending the respiratory tract against infections and maintaining its health. It consists of various components, including immune cells, antibodies, and cytokines (such as IL-13), which regulate immune responses. When the respiratory system is exposed to pathogens or irritants, the immune system responds by triggering inflammation and activating immune cells to fight off the invaders (Yuksel *et al.*, 2023).

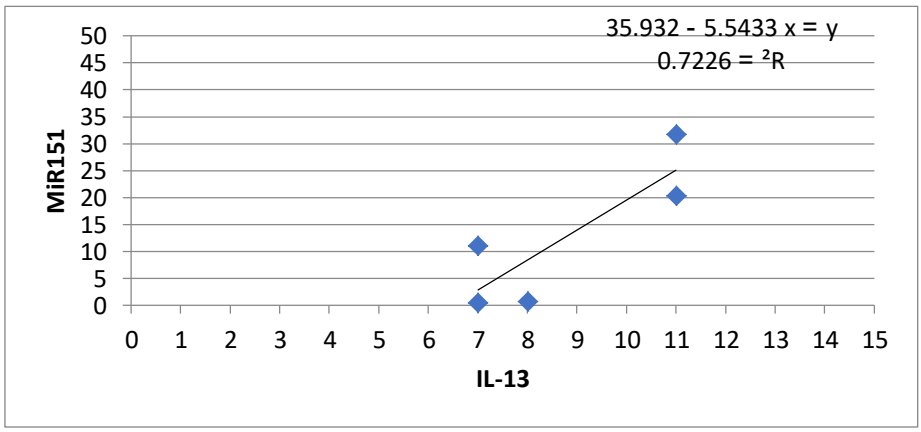


Figure (4.4): Relationship between IL-13 and MiR151 in respiratory diseases.

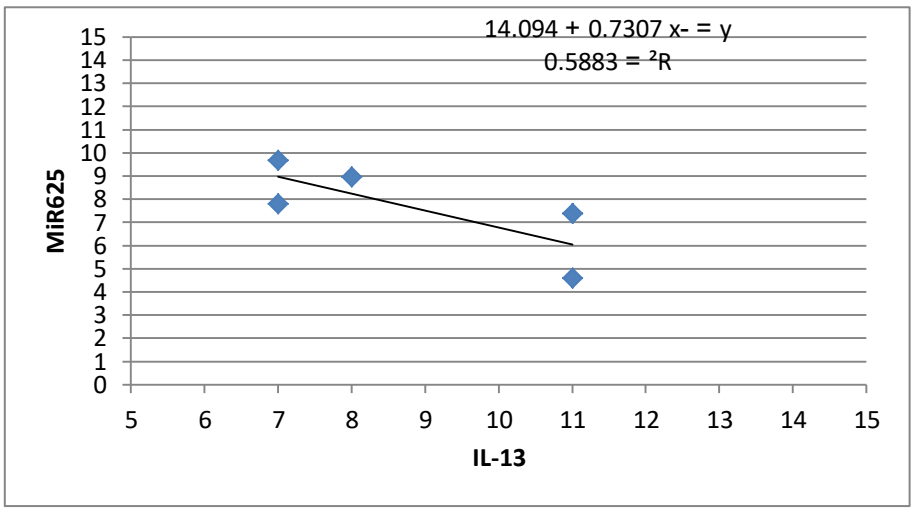


Figure (4.5): Relationship between IL-13 and MiR625 in respiratory diseases.

Regarding miRNA-151, miRNA-625, and IL-13, their specific roles in bronchitis are not well understood. However, research has indicated potential involvement in respiratory diseases, including bronchitis, asthma, and COPD (Qian *et al*., 2018; Han *et al*., 2020; Fathollahpour *et al*., 2023). miRNA-151, as mentioned earlier, is a small RNA molecule involved in gene regulation. Although its specific role in bronchitis is unclear, studies have implicated miRNA-151 in inflammatory processes and immune response modulation. It may have the potential to influence the expression of genes related to inflammation or immune regulation in the bronchial tubes (Guo *et al*., 2020). miRNA-625 exact Similarly role in bronchitis is not well characterized. However, it has been associated with various inflammatory conditions and cancers. It's possible that miRNA-625 could be involved in regulating immune responses or inflammatory processes in the respiratory system, potentially impacting bronchitis (Yang *et al*., 2011).

Interleukin-13 (IL-13) is a cytokine that plays a significant role in asthma and other respiratory diseases. It promotes inflammation, mucus production, and airway remodeling, contributing to the pathogenesis of bronchitis. Elevated levels of IL-13 have been observed in the bronchial tubes of individuals with chronic bronchitis and asthma, suggesting its involvement in these conditions (Fulkerson *et al*., 2006).

5. Conclusions and Recommendations

5.1 Conclusions

1-microRNA-625 and microRNA-151 play important roles in the regulation of IL-13 mediated airway inflammation, remodelling, and microbial infection in respiratory
disorders.

2-Targeting microRNA-625 and microRNA-151 could provide novel therapeutic strategies for the treatment of IL-13-driven respiratory diseases and microbial infections.

3-Further research is needed to validate the potential of these microRNAs as biomarkers and therapeutic targets

5.2 Recommendations

1- Explore the potential of targeting IL-13, microRNA625, or microRNA 151 as therapeutic strategies for respiratory disorders.

2- Conduct clinical studies to assess the expression levels of IL-13, microRNA625, and microRNA 151 in patients with respiratory disorders and correlate these findings with disease severity and outcomes

3-Utilize appropriate animal models of respiratory disorders to study the role of IL-13 microRNA625, and microRNA 151 in disease pathogenesis.

Supplements

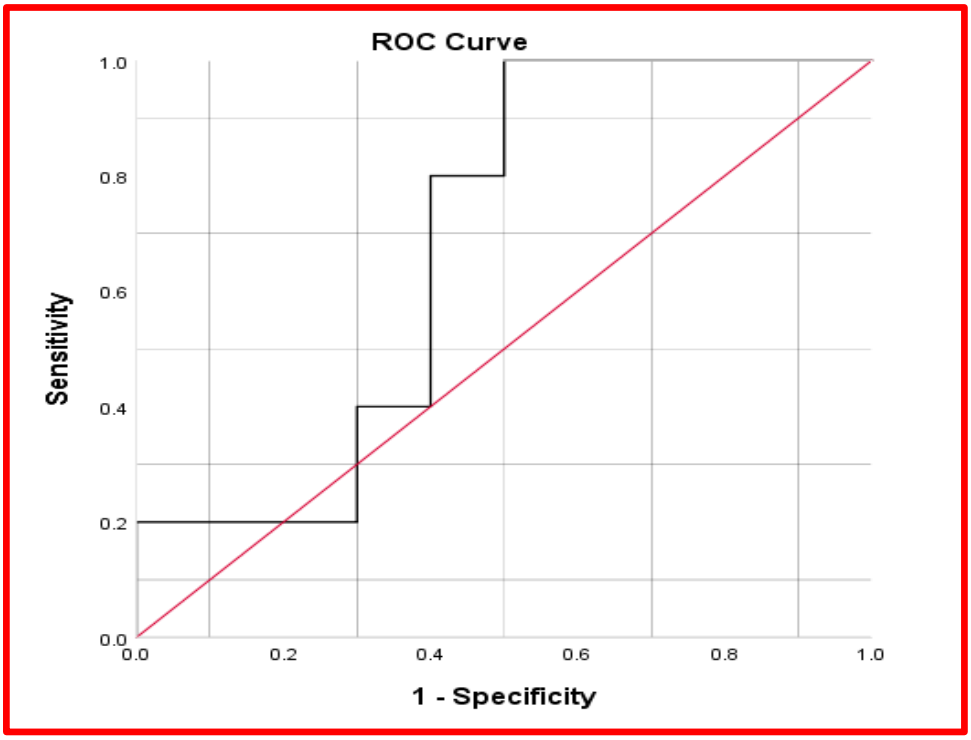


Figure (1): ROC curve for prediction of the disease activity by MiR151 in Sensitive bronchitis

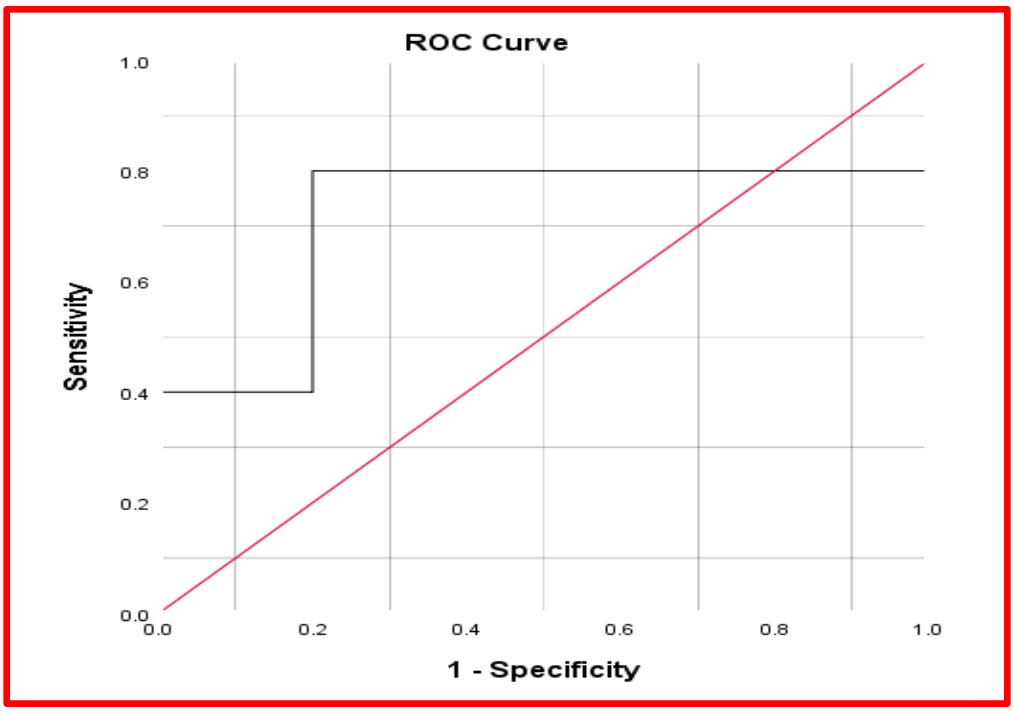
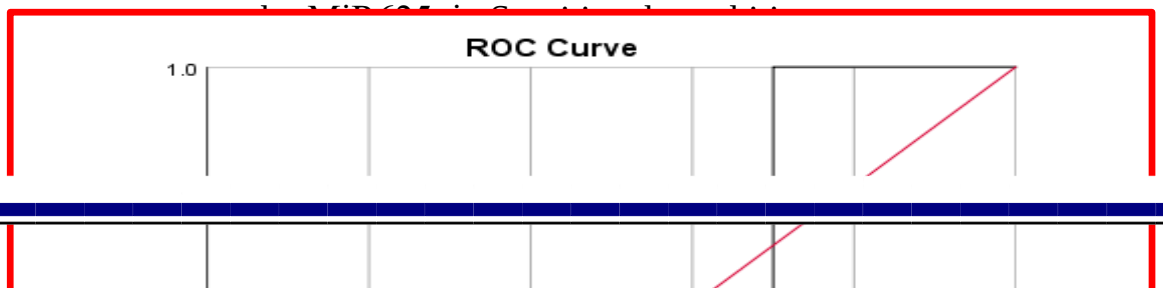


Figure (2): ROC curve for prediction of the disease activity



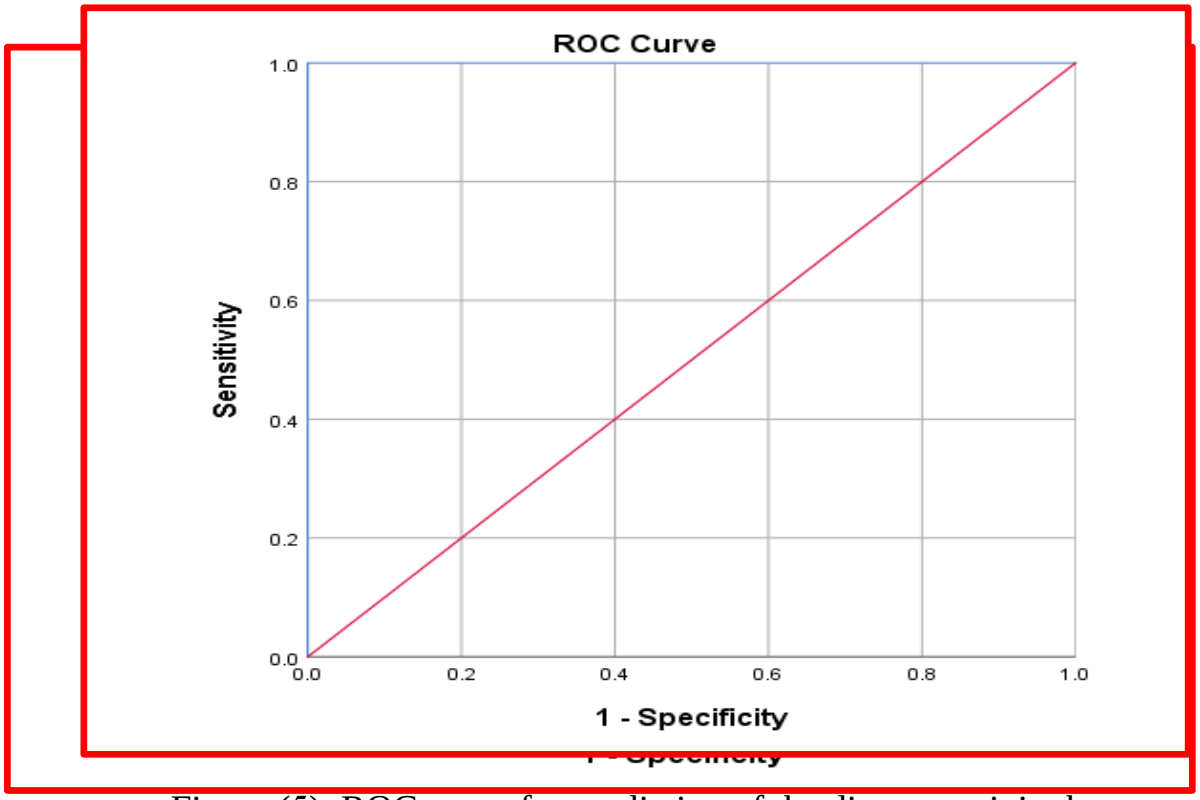


Figure (5): ROC curve for prediction of the disease activity by MiR151 in Bronchitis.

Figure (4): ROC curve for prediction of the disease activity by MiR625 in Asthema.

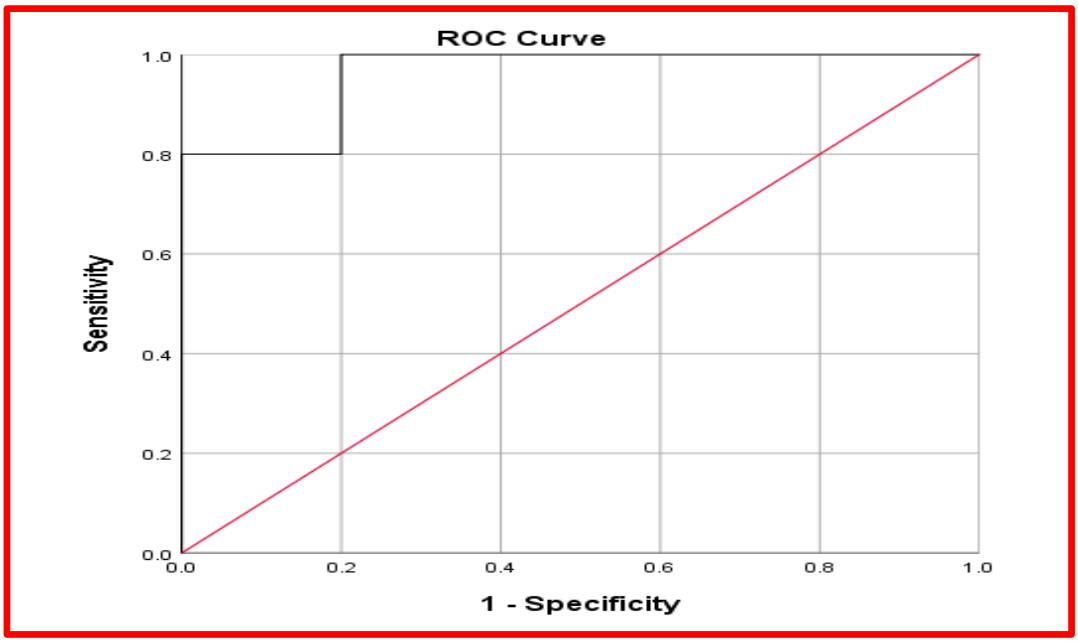
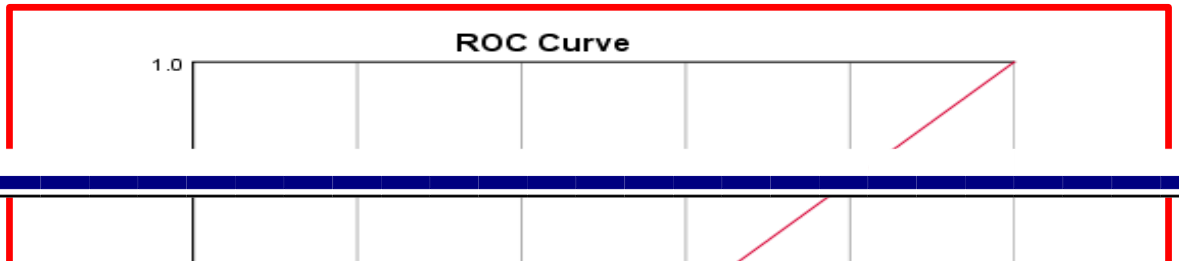


Figure (6): ROC curve for prediction of the disease activity by MiR625 in Bronchoititis .



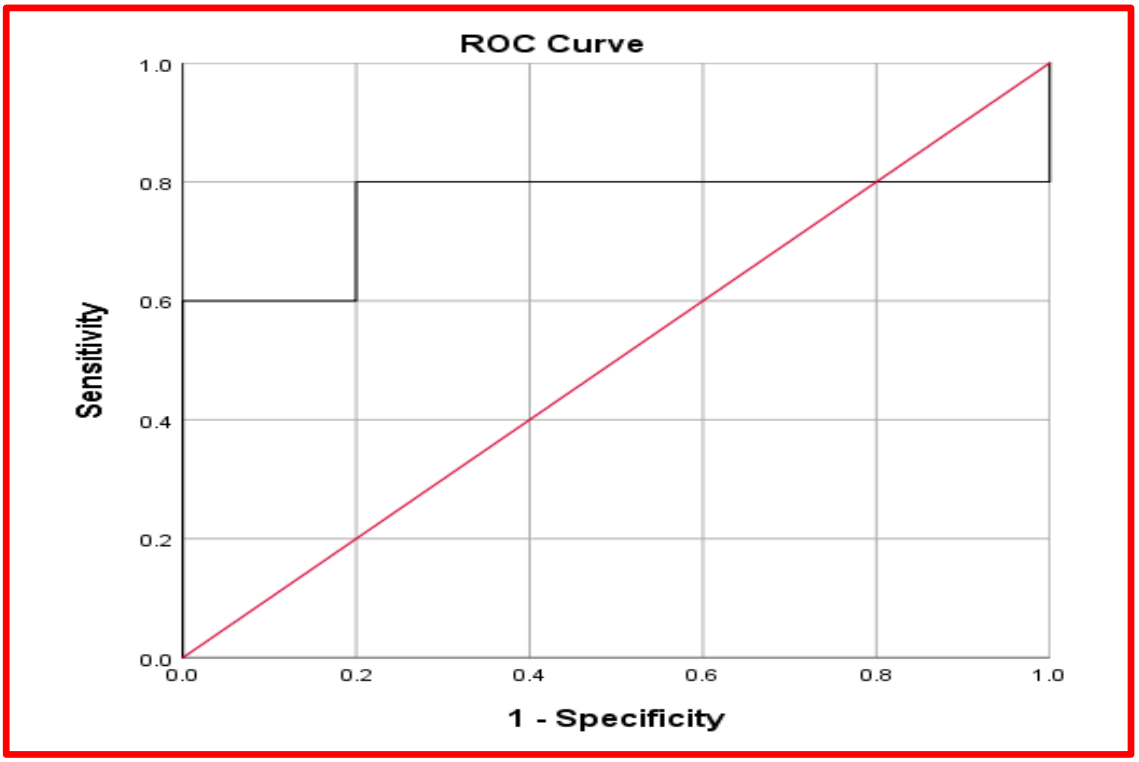
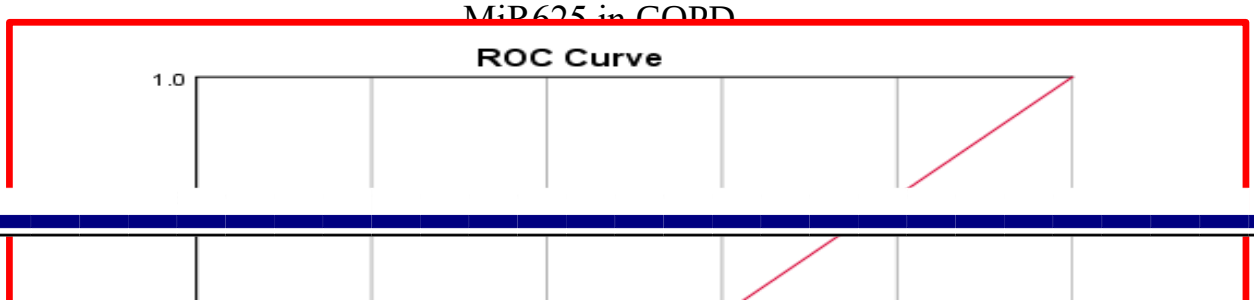


Figure (8): ROC curve for prediction of the disease activity by MiR625 in COPD



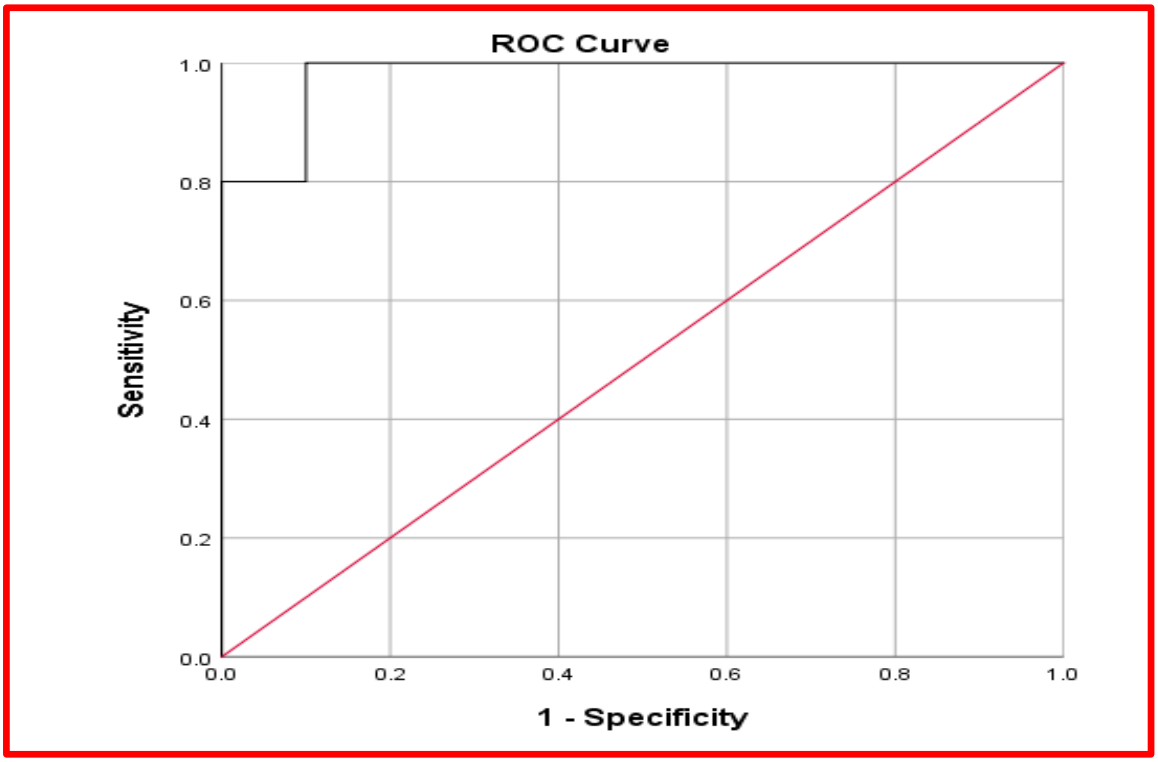
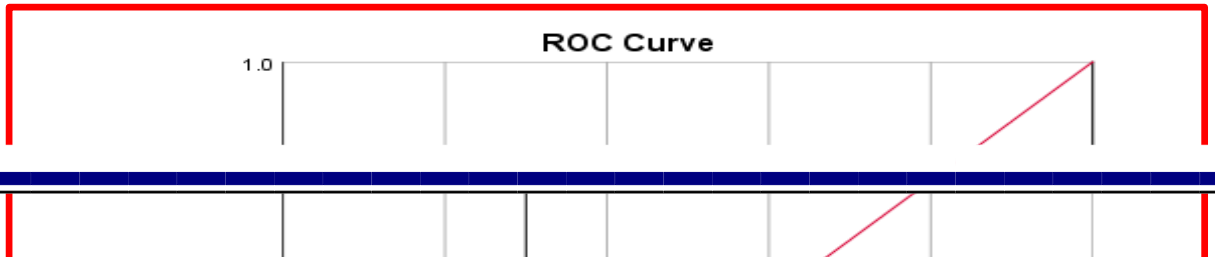


Figure (10): ROC curve for prediction of the disease activity by MiR625 in covid-9.



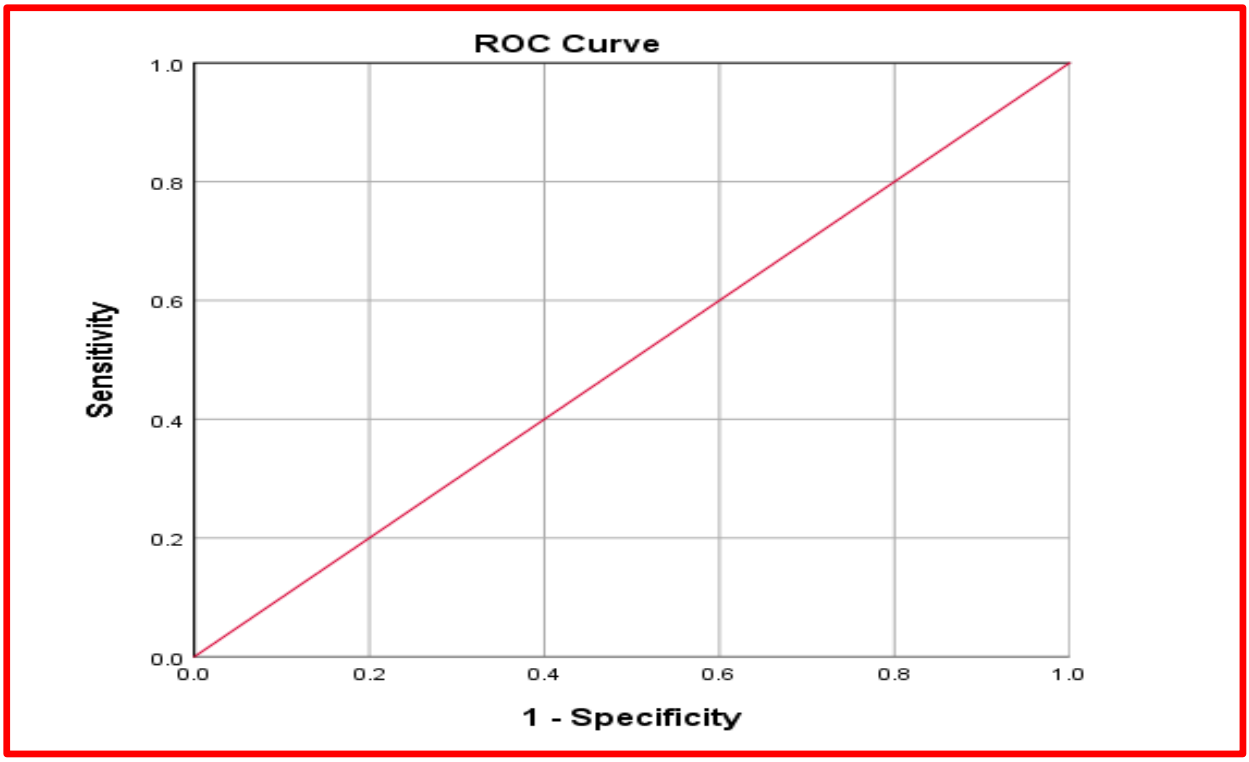


Figure (12): ROC curve for prediction of the disease activity by MiR625 in TB.

Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	U 22 MiR151 FAM:	U 23 MiR151 FAM:	U 24 MiR151 FAM:	U 25 MiR151 FAM:	U 26 MiR151 FAM:	U 27 MiR151 FAM:	U 28 MiR151 FAM:	U 29 MiR151 FAM:	U 30 MiR151 FAM:	FAM:	FAM:	FAM:
B	U 31 MiR151 FAM:	U 32 MiR151 FAM:	U 33 MiR151 FAM:	U 34 MiR151 FAM:	U 35 MiR151 FAM:	U 36 MiR151 FAM:	U 37 MiR151 FAM:	U 38 MiR151 FAM:	U 39 MiR151 FAM:	FAM:	FAM:	FAM:
C	U 22 U6 FAM:	U 23 U6 FAM:	U 24 U6 FAM:	U 25 U6 FAM:	U 26 U6 FAM:	U 27 U6 FAM:	U 28 U6 FAM:	U 29 U6 FAM:	U 30 U6 FAM:	FAM:	FAM:	FAM:
D	U 31 U6 FAM:	U 32 U6 FAM:	U 33 U6 FAM:	U 34 U6 FAM:	U 35 U6 FAM:	U 36 U6 FAM:	U 37 U6 FAM:	U 38 U6 FAM:	U 39 U6 FAM:	FAM:	FAM:	FAM:
E	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:
F	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:
G	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:
H	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:	FAM:

Figure (13): qPCR

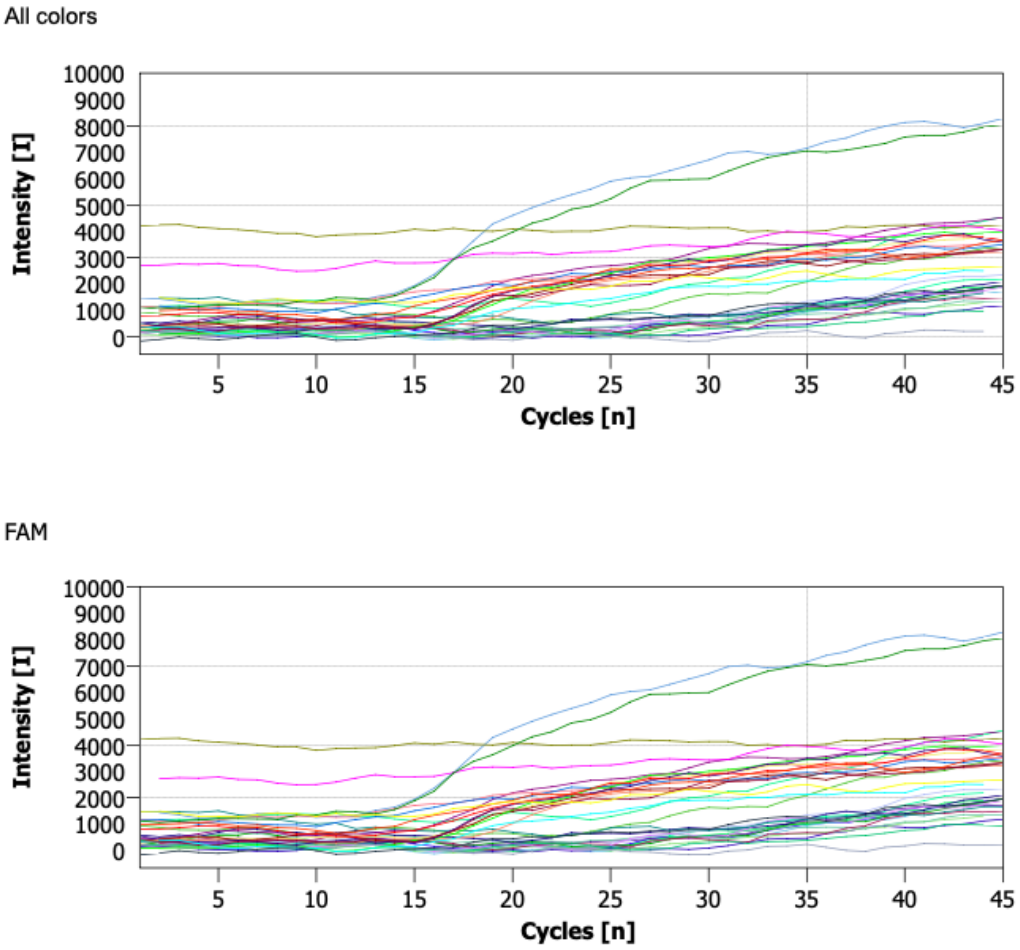


Figure (14): Monitoring - Raw Data

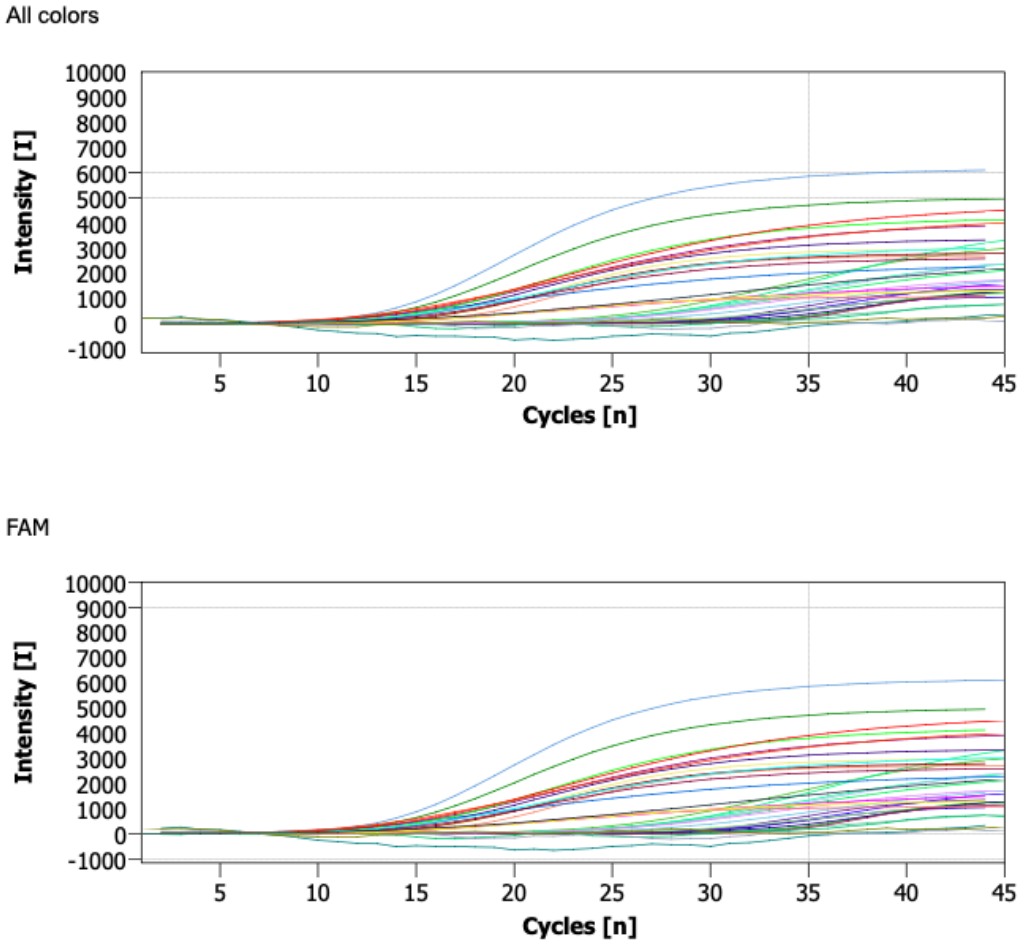


Figure (16) Monitoring - Raw Data

خلاصة:

يشير اضطراب الجهاز التنفسي إلى مجموعة واسعة من الحالات التي تؤثر على أمراض الجهاز التنفسي العلوي والسفلي. يشمل الجهاز التنفسي العلوي التهاب الجيوب الأنفية ونزلات البرد ولسان المزمار والتهاب البلعوم، وتشمل أمراض الجهاز التنفسي السفلي الربو وفيروس كورونا والسل والالتهاب الرئوي. سبب أمراض الجهاز التنفسي هو البكتيريا والفيروسات والفطريات والحالة البيئية.

الهدف من الدراسة هو تحديد تركيز IL-13 باستخدام تقنية Elisa، وتقدير بعض أدوار microRNA 151,625 في أنواع مختلفة من أمراض الجهاز التنفسي باستخدام RT QPCR ومعرفة أنواع البكتيريا المؤثرة على الجهاز التنفسي. تم جمع 155 عينة تنوعت بين مصابة وأصحاء في المحاويل ومستشفى مرجان التعليمي ومركز المختار لمرض السل في محافظة بابل/ العراق. خلال الفترة من ايلول 2022 إلى شباط 2023. تم تقسيم العينات على النحو التالي: تم جمع 107 عينة من مريض يعاني من اضطراب في الجهاز التنفسي. تم توزيع العينات على بعض الأمراض بما في ذلك (الربو، كوفيد، التهاب الشعب الهوائية، التهاب الشعب الهوائية الحساسة ومرض الانسداد الرئوي المزمن) معايير استبعاد مريض مصاب بأمراض أخرى مثل مرض السكري والتهاب المفاصل وضغط الدم واضطرابات الكلى وأمراض القلب ب. تم جمع 48 عينة من حالة مستقرة ظاهريا المجموعة الضابطة المصابة بأمراض الجهاز التنفسي.

أظهرت نتيجة اختبار الاليزا تركيزا عاليا من IL-13 في مرض السل (14.5 ± 0.8) وتركيز منخفض في الربو (4.0 ± 1.70) مقارنة مع عينات من الاصحاء (9.3 ± 1.4)، أظهر الحمض النووي الريبي (625 جين) تعبيرا جينيا اقوى في مرض السل (9.15 ±1.1)، وأظهر انخفاض التعبير الجيني في التهاب الشعب الهوائية (2.03 ± 0.4) مقارنة مع الاصحاء (420 ± 0.2).

(151 جين) أظهر ارتفاع الجين في مرض الانسداد الرئوي (COPD) (35.32 ± 1.1) وانخفاض التعبير الجيني في التهاب الشعب الهوائية الحساس (4.26 ± 2.43) مقارنة مع السيطرة (1.64 ± 0.5). أعلى نسبة من strptococcus pneumonia ونسبة منخفضة من بكتيريا Klebsiella pneumonia وتوصلت هذه الدراسة إلى وجود علاقة بين IL-13 وMiRNA (625,151) مع اضطراب الجهاز التنفسي.**وهذا يعني انه عندما تكون العلاقة بين MIRNA(625-151) وIL-13 ايجابية ممكن استخدام MIRNA كتشخيص للمرض وعندما تكون العلاقة بين miRNA وIL- 13 سلبية ممكن استخدامه كعلاج .**

وزارة التعليم العالي والبحث العلمي
جامعـــــــــــة بابـــــــــــل
كلية العلوم للبنات قسم علوم الحياة

دور MicroRNAS في اضطرابات الجهاز التنفسي: استكشاف العلاقة بين IL-13 و MiRNA-625 و MiRNA-151 والعدوى المكروبية

رسالة مقدمة الى
مجلس كلية العلوم للبنات – جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل الطالبة

رنا كاظم كريم المعموري

بكالوريوس علوم حياة – جامعة بابل

سنة التخرج 2004-2003

اشراف

الاستاذ الدكتور

عبد النبي جويد المعموري

١٤٤٤هـ ٢٠٢٣م

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