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Evaluation of Some Immunological and Molecular Markers in Patients with Bacterial Tonsillitis Infections

A Thesis

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By

Zeina Ali Hussain Ali

(B.Sc. in Science for Woman of Biology / University of Babylon 2016)
(M.Sc. in Science for Woman of Biology / University of Babylon2019)

Supervised by

Prof. Dr. Azhar Omran Al-Thahab

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

رَبَّنَا لَا تُزِغْ قُلُوبَنَا بَعْدَ إِذْ هَدَيْتَنَا
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Certification

This is to certify that the preparation of this **dissertation** entitled (**The Association of Some Cellular Immunity Parameters among Patients with Tonsillitis Infection**) was made under my supervision by (**Zeina Ali Hussain Ali)** and submitted in partial fulfillment of the requirements for **the Degree of Doctor of Philosophy in Biology/Microbiology** presented to **Department of Biology / College of Science / University of Babylon.**

Signature:
Name: **Prof. Dr. Azhar Omran Al-Thahab**
Address: Department of Biology College of Science University of Babylon
Date: / /2023

In view of the available recommendation. I forward this **thesis** for debate by the examination committee.

Signature:
Name: **Assist. Prof. Dr. Adi Jassim Abd AL-Rezzaq**
Address: Head of Biology Department, College of Science
University of Babylon
Date: / / 2023

Dedication

To my mother…

Who prays for me every day …

My mother, who supported me with every detail of my life, the first and last supporter, is a light for the darkness of my life …

To my father…

Who is supportive and safe in every step …

To My teacher and my spiritual father Dr-Adnan Hashim

To my sister Dina and my brothers…

Who support me with everything my dearest friend Ruqia fadhel…

To my husband's Mohaeman soul and life partner

To my country …to Iraqi martyrs … to all the free people

I dedicate this simple work wishing that it participates in Iraq development.

Zeina-2023

Acknowledgement

I would like to express my thanks to "**Allah**" the most gracious and most merciful, and to his prophet "**Mohammad**", god's blessings and Peace be upon Mohammed and his family for enabling me to present research.

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Summary

Tonsillitis is a common and widespread disease and inflammation of the tonsils in the pharynx. a common condition caused by Several organisms, including bacteria, viruses, yeast, fungi, and parasites, can cause tonsil inflammation .The current study included an evaluation of some immune and cellular parameters at the level of systemic and local immunity for patients with acute ,chronic and recurrent tonsillitis caused by Gram-positive and Gram-negative bacteria for the period from March to September 2022. The study included 90 patients, including 51 males and 39 females, who visited Imam Hassan Al-Mujtaba (peace be upon him) Hospital. Educational and some outpatient clinics in Karbala suffer from tonsillitis. They were diagnosed by an ear, nose and throat specialist, and 45 people of the same ages were considered a healthy control group The number of males was 28 and females were 17 all specimen age group between (1_60)year

For all patients and healthy people, a blood sample was taken (with and without an anticoagulant) for immunological and molecular study. As well as two tonsil swabs for each patient, one of which is placed in a sterile saline solution for the purpose of local immunity study, and the other is placed in a solution containing culture medium for the microbial culturing .

The bacteria swabbed from the tonsils were diagnosed and stained with gram stain to determine if they were positive or negative after cultivating them on a group of culturing media. The diagnosis was also confirmed by the Vitek method to determine the type of bacteria common in the disease.

The most common Gram-positive bacteria were *Streptococcus pyogenes* 24/90(26.6%), while the most common Gram-negative bacteria

were *Klebsiella pneumonia* 11/90(12.2%).The type of infection can classified acute48\90(53.3%), Chronic 37/90(41.1%) and Recurrent 5/90(5.6%)

The Enzyme Linked Immune Sorbent Assay (**ELISA)** test was conducted to determine the concentrations for all samples after taking two samples for each person, one of which was topical, consisting of swabs swabbed from the tonsils and placed in a saline solution, and the second was systemic, which was serum. After placing the blood sample in an anticoagulant and using a centrifuge, the result was that CD4, CD19, and IL9 increased. The concentration in patients and healthy people increased both locally and systemically, as the concentration of CD4 in patients was (22.11±3.66) pg/m and (17.39±1.38) pg/m in healthy people systemically, and the concentration in patients was (21.97±1.53) pg/m and (15.76±1.97) pg/m in healthy people topically, while the concentration of CD19 in In patients (49.34±3.57) ng/m and (22.81±6.59) ng/m in healthy people systemically, the concentration in patients was (47.33±2.91) ng/m and (25.9±3.83) ng/m in healthy people locally, while the concentration of IL9 in patients was (13.07±4.92) pg/m and (10.74±4.66) pg/m in healthy people systemically, the concentration in patients was (12.91 ± 3.35) pg/m and (10.18 ± 3.94) pg/m in healthy people topically, in contrast to IL3, which decreased in patients and increased in healthy people locally and systemically, where the concentration of IL3 in patients was (9.119 ± 2.66) pg/m and (11.31 ± 2.98) pg/m in healthy people. Systemically, the concentration in patients is (5.69 ± 1.107) pg/m and (8.76 ± 2.181)pg/m in healthy people topically.

Gene polymorphisms were investigated for all patients by the allele-specific method using the gene-specific primer, and work was done on two genes, *IL-3* (rs40401) genotypes and alleles. First codominance mode of inheritance, there was significant variation in genotype

distribution of wild CC in such a way that the heterozygous genotype CT distribution were more frequent in the patients group but the homozygous genotype TT non -significant variation in genotype distribution ,*IL-9* (rs2069870). genotypes and alleles. First co dominance mode of inheritance, there was significant variation in genotype distribution of wild AA in such a way that the heterozygous genotype AG distribution were more frequent in the patients group but the homozygous genotype GG non-significant variation in genotype distribution.

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List of Abbreviations

Abbreviation	Term
AT	**Acute tonsillitis**
AS	**Ankylosing spondylitis**
ANA	**Anti- nuclear antibodies**
Abs	**Antibodies**
Apcs	**Antigen presenting cell**
Ags	**Antigens**
BHS	**Beta haemdytic *Streptococcus***
BMDMs	**Bone marrow cell differentiated**
CT	**Chronic tonsillitis**
CD 8	**Cluster differentiation**
CD4 Tall	**Cluster differentiation 4 T cell**
CD19	**Cluster of differentiation 19**
CD4	**Cluster of differentiation 4**
CSF	**Colony stimulating factor**
DC	**Dendritic cells**
DNA	**Deoxyribonucleic acid**
ENT	**Ear, nose and throat**
ELISA	**Enzyme Liked Immuno Sorbent Assays**
EBV	**Epstein barr viruss**
EDTA	**Ethylene di amine tetra acetic acid**
Gc	**Germinal center**
GABHS	**Group A beta- hemolytic *streptococcus***

GAS	**Group A *streptococcus***
HA	**Hemagglutinin**
HRP	**Horse radish peroxidase**
HIV	**Human immunodeficiency virus**
HLA II	**Human leukocyte antigen**
IFN	**Interferon**
IL3	**Interleukin 3**
IL9	**Interleukin 9**
IL10	**Interleukin 10**
IL 17	**Interleukin 17**
IL6	**Interleukin 6**
MHCII	**Major histo compatibility complex type II**
MZ	**Marginal zone**
mRNA	**Messenger ribo nucleotide amino acid**
MALT	**Mucosa associated lymphoid tissue**
NLR	**Neutrophile to lymphocyte ration**
OSA	**Obstructive sleep apnoea**
OR	**Odds ratio**
OTUS	**Operational taxonomic units**
OD	**Optical density**
pAMPs	**Pathogen associated molecular patterns**
PASA	**PCR amplification of specific alleles**
PTA	**Peritonsillar acute**
PCR	**Polymerase chain reaction**

RBCs	**Red blood cells**
RA	**Rheumatoid Airthritis**
SNP	**Single Nucleotide Polymorphism**
SEB	***Staphylococcal* enterotoxin B**
SLE	**Systemic lupus erythematosus**
TCR	**T cell antigen receptors**
TCR	**T cell receptor**
TFH	**T follicular helper**
Th 1	**T helper 1**
Th 2	**T helper 2**
Th 17	**T helper17**
T reg	**T regulatory**
TLR 4	**Toll like receptor 4**
TLR 8	**Toll like receptor 8**
TBE buffer	**Tris – borate EDTA**
TNF	**Tumor necrosis factor**
URT	**Upper respiratory tract**
WT	**Wild type**

Chapter One

Introduction

1-1 Introduction

The tonsils are two lymph nodes located at the back of the throat on either side. They serve as a defense mechanism, preventing infection from entering the body. Tonsillitis is simple to identify and treat (Shantinath and Sajane, 2015).

Several organisms can stimulate inflammation of the tonsils, these include bacteria, viruses, yeasts, fungi and parasites, few of the infectious organisms are part of the normal oropharyngeal flora whereas others are external pathogens. Because the oropharynx is colonized by many organisms, some infections are polymicrobial; these organisms work synergistically and can be demonstrated in mixed aerobic and anaerobic infections (Stelter, 2014). Tonsils are important organs of the human immune system that protect the body from pathogens invading the upper respiratory tract, particularly in young children (Brambilla *et al*., 2014). Tonsils are involved in both humeral and cellular immunity (Zielnik-Jurkiewicz, ; 2002). The tonsils contain both B and T lymphocytes, which are involved in both humeral and cellular immunity (Brandtzaeg, 2013).

Over 80 million autosomal single nucleotide polymorphisms (SNPs), including 8 million common variants, have been discovered in genomic surveys of individuals from multiple populations (Auton *et al*., 2015). Genome-wide association studies (GWAS) have identified disease risk associations for thousands of these SNPs (Visscher *et al*., 2012).

PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP or small deletions in a sequence See SNP genotyping for more information (Bulduk *et al.,* 2020).

A tetra-primer ARMS-PCR method to detect a single leptin mutation using genomic DNA collected from mice's tails. Tetra-primer ARMS-PCR has been applied to identify the single nucleotide polymorphism (SNP) (Islam *et al*., 2021) and differentiate whether the single mutation in DNA is heterozygous or homozygous (Komijani *et al*., 2022). This method takes advantage of the relative inability of Taq DNA polymerase to extend primers mismatched at their 3′-end and its intrinsic propensity of lacking 3′ to 5′ exonuclease activity, so the extension is dramatically reduced (Vamvakopoulos, 2002)

1-2: Aim of study

Due to the prevalence and frequency of respiratory infection and its recurrence, the current study was designed to shed light on it is through some stimulating cellular immunological criteria and its genetic relationship to the disease through the following steps:

1- Diagnosis of the most important bacterial causes in different ways such as: culture characteristic, biochemical test and vitek 2 system.

2- Measurement and determination some immunological parameters such as: IL3, IL9 ,CD4 ,CD19 concentration by ELISA

3- Detection polymorphism gene for *IL-3* and *IL-9* by allele specific PCR method .

4- Found the relationship between cytokine concentration and genes polymorphism.

5- Compared between mucosal and systemic cytokine concentration

Chapter Two

Literatures Review

2-1 History of tonsillitis

Tonsillitis, an inflammation of the tonsils brought on by bacterial or viral infection, is a frequent clinical disorder (Kalaiarasi *et al*.,2018). It affects a substantial portion of the populace, particularly kids. When a person experiences seven or more acute tonsillitis attacks each year, they are said to have chronic tonsillitis (CT) (Mbalaso ,2015).

Between the anterior palatoglossal arch and the posterior palate pharyngeal arch, in the lateral oropharynx, are the tonsils in 70% to 95% of cases, viral infections are the cause of Rhinovirus, respiratory syncytial virus, adenovirus, and coronavirus are some of the most prevalent viral causes. Epstein-Barr virus, CMV, hepatitis A, and rubella are other less engage in sexual activity should be tested for syphilis, gonorrhea, chlamydia, and HIV. Recurrent tonsillitis may result from tuberculosis (Anderson and Paterek, 2022) Only 50 to 70 percent of tonsillitis patients have a pathogen that can be identified ((Windfuhr *et al*.,2016).

One of the most frequent surgical procedures carried out by ENT doctors is tonsil surgery. The distinctions between therapy approaches, including those related to indications, are still being researched. Despite a decline in the number of cases requiring surgery owing to infection, a considerable percentage of cases continue to have an infectious cause (Hallenstål *et al*.,2017). The primary benefit of the TT approach is the maintenance of the tonsil capsule, which acts as a biological bandage, shielding the pharyngeal muscles from direct trauma and inflammation brought on by saliva contact. As a result, recovery time and postoperative pain are both decreased(Kordeluk *et al* .,2016).

Virus or bacterial infections are typically the cause of symptoms. Twenty to thirty percent of pediatric hospitalizations and thirty to sixty percent of pediatric outpatient visits are for respiratory tract infections. Upper respiratory infections are primarily brought on by acute tonsillitis and/or pharyngitis, which are typically secondary to viruses and bacteria. *S. pyogenes*, a group A *streptococcal* (GAS) illness, is the most prevalent and significant cause of bacterial tonsillopharyngitis. It can cause both early and late sequelae, including acute rheumatic fever and post-streptococcal glomerulonephritis (Gurol *et al*., 2017).

2-2 Epidemiology of tonsillitis

A common respiratory tract condition is tonsillitis. It affects both children and adults, is common, has detrimental social, professional, and economical effects, and is brought on by infection with bacteria, viruses, and allergens can produce inflammation by interacting with the lymphatic tissue in the tonsils (Nabat *et al* ., 2019).

Each year, around 9 million new cases of tonsillitis are identified. (Haidara *e.t al*., 2019). Tonsil irritation is a frequent condition that accounts for 1.3% of outpatient visits (Kocher and Selby 2015). It primarily manifests as a sore throat when it is uncomplicated and is caused by a viral or bacterial infection(Bartlett *et al*.,2015). Tonsillitis inflammation, usually with a sudden onset. It is a specific kind of pharyngitis. A sore throat, fever, enlarged tonsils, difficulty swallowing, and swollen lymph nodes in the neck are a few symptoms that can occur. One of the complications is peritonsillar abscess (Klug *et al*., 2016)

Less frequent symptoms include nodes in the neck, discomfort in the ears or neck, nausea, stomach pain, vomiting, a furry tongue, foul

breath, a change in voice, and trouble opening the mouth(Brandtzaeg 2011).The study was to determine the most common bacterial pathogens that cause acute tonsillitis, as well as how sensitive they were to antibiotics, in order to determine the best course of treatment and prevent acute tonsillitis complications and avoid unnecessary surgical procedures (Chan *et al*., 2013). Pharyngeal tonsils become inflamed when someone has tonsillitis. The adenoids and lingual tonsils, as well as other regions in the back of the throat, may be impacted by the inflammation. They normally begin to show up around the fourth or fifth month of pregnancy and continue to grow along with the baby (Chiappini *et al* ;2012). Tonsils are present at birth, and they typically attain their full size between the ages of 6 and 8. Between the fourth and twelfth years of life, tonsils and adenoid tissue are discovered to be the most immunologically active, and shortly after the first decade, they start to involute/atrophy(Cingi *et al* ;2010).

Recurrent tonsillitis and sore throats affect many kids so frequently that they develop accustomed to them. For instance, according to one study, tonsillectomies are necessary for about 30% of peritonsillar abscesses (Herzon and Harriy .,1995) .and another shows that youngsters in Norway and Turkey, respectively, report having recurrent tonsillitis at rates of 11.7% and 12.1% (Kvestad *et al*.,2005) .Many of these individuals are given antibiotic prescriptions, which often only offer momentary respite until the tonsillitis returns (Ward , 2018) . Researchers at Washington University School of Medicine discovered that the development of biofilms by microorganisms in the moist, warm folds of the tonsils, which serve as a store of infection, worsens recurring infections(Chole and Faddis ,2003).

Biofilms were found in 70.8% of chronic tonsillitis patients, according to a study that used an advanced imaging technology on small pieces of human mucosal tissue (Kania *et al*.,2007). Another study discovered that biofilms were present on the surface epithelium of the tonsils and adenoids in many of the patients who were awaiting for adenotonsillectomy due to chronic tonsillitis and adenoiditis (Al-Mazrou and Al-Khattaf ,2008). These biofilms are also seen in other otorhinolaryngology-related illnesses, including chronic rhinosinusitis and chronic otitis media with effusion(Saylam *et al*.,2010).

2-3 Anatomy of the palatine tonsil

The tonsillar fossa, which houses the lymphoid structure known as the palatine tonsil, is bordered on the anterior and posterior sides by mucosal folds that are made up of the palatoglossus and palatopharyngeus muscles, respectively. The tonsillar fossa's lateral boundary is created by the superior constrictor muscle. The buccopharyngeal fascia, the last line of defense between the tonsil and the parapharyngeal area, and a layer of loose connective tissue are located deep to this muscle(Krishnan , 2020)

Several external carotid artery branches that travel via the superior pharyngeal constrictor muscle supply blood to the tonsil. The tonsillar artery, a facial arterial branch, mostly serves the inferior pole. The inferior pole receives additional blood flow from the lingual artery's dorsal lingual branches. The ascending palatine artery, another branch of the facial artery, divides distally into two branches, one of which also supplies the tonsil in the palatine area. The superior pole of the palatine tonsil is supplied by the ascending pharyngeal artery's tonsillar branches and the descending palatine artery, respectively. one of the branches of the internal maxillary artery. Venous drainage is carried out through the

paratonsillar vein, which afterwards connects to the common face vein and the pharyngeal venous plexus. Lymphatic drainage basins include the jugulodigastric lymph nodes and, infrequently, the retropharyngeal lymph nodes. The glossopharyngeal nerve's tonsillar branches and the lesser palatine nerve from the second division of the trigeminal nerve (V2) supply sensory information to the palatine tonsil and tonsillar fossa. Because the tympanic nerve branch of the glossopharyngeal nerve also transmits sensation to the middle ear, patients with tonsillar disease or those who have just undergone tonsillar procedures can experience referred otalgia (Shah and Garritano,2015).

2-4 Causes of tonsillitis

The most common causes of tonsillitis are bacterial, particularly beta hemolytic and other streptococci; however, in tonsillitis caused by infectious mononucleosis, the most common virus is EBV, which is present in 50% of children. Infections with Cyto-Megalovirus (CMV), Hepatitis A, Human Immunodeficiency Virus (HIV), Rubella, and Toxoplasmosis can also result in the clinical picture of infectious mononucleosis, necessitating a differential diagnosis (Georgalas *et al*.,2009). *S.aureus* appears to be the primary pathogen in the pathogenesis of acute tonsillitis (Mahajan and Ingale. 2017).

2-4-1 Bacterial causes of tonsillitis

The human body is affected by oral bacteria, which have a strong link to disease. According to various studies, there are correlations between these areas' microbe populations, which migrate from the mouth to the alimentary tract at a rate of about $8*10^{11}$ bacterial cells each day(Segata ;2012 ; Gao.,2018) .Despite the fact that *Porphyromonas gingivalis* can change intestinal microflora and a variety of oral germs can

enter the gut microbiota, only certain of these bacteria populate the gut under dysbiotic conditions (Olsen & Yamazaki, 2019). The mouth cavity shows a higher abundance of the core operational taxonomic units (OTUs) shared by more than 95% of subjects than the rest of the body, according to microbiome research at 18 body regions of 200 people(Huse *et al*.,2012).

Infections caused by bacteria and fungi are more serious. Group A *Streptococcus* (GAS) infection caused by *Streptococcus pyogenes,* also known as "strep throat," affects up to 30% of children and 15% of adults, respectively, when they have sore throats (Nakhoul and Hickner,2013 ; Cars *et al*.,2017).

Acute rheumatic fever, glomerulonephritis, and pediatric autoimmune neuropsychiatric disorders linked to streptococcal infections are examples of nonsuppurative complications. Vincent angina, peritonsillar abscess, and septic jugular-vein thrombophlebitis are examples of suppurative complications that require immediate medical attention or surgery(Sigra *et al*.,2018).

Several different types of bacteria can be found in the microbiota of the various URT locations. The most often isolated genera include *Streptococcus*, *Neisseria*, *Haemophilus*, *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Prevotella*, and *Porphyromonas*(Wilson, 2010 ; Clark, 2020). The most prevalent among them are Haemophilus influenzae, Moraxella catarrhalis, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. (Clark, 2020 ; Claassen-Weitz *et al*.,2021) . The increase in invasive pathogenic bacteria, which causes infection and inflammation, is linked to the imbalance in the URT microbiota. After pathogenic bacteria colonize the mucous membrane of

the URT, infection and disease progression take place (Bellussi *et al*.,2019).

Group A beta hemolytic streptococcus (GABH S), also known as *Streptococcus pyogenes*, is the most significant culprit causing tonsillitis. It is a human pathogen that causes a wide spectrum of infections and contributes significantly to worldwide morbidity and mortality (Muhtarova *et al*.,2020).

In order to study the oral cavity microbiota, samples of mouthwash are frequently collected .and its composition has been linked to conditions including oropharyngeal and oral malignancies (Wang *et al*.,2017; Rosenbaum *et al*.,2019).

Streptococcus pyogenes group The most typical bacterial cause of pharyngitis for which antibiotics are recommended is a streptococcus (GAS). The *emm* gene encodes more than 240 different M protein types, each of which is associated with a particular *emm* type. Certain *emm* types have long been linked to side effects like rheumatic fever (type 1, for example) and acute glomerulonephritis (type 12, for example)(Shulman and Bisno 2012,)

Group A *streptococcus* GAS has a brief incubation period of 2–5 days and is transmitted from person to person through respiratory droplets. When kids spend most of their time indoors for school and sports in the late winter and early spring, GAS pharyngitis is at its worst. Even though up to 20% of school-age children may have GAS in their throats during the winter months, colonization has not been proved to have an impact on the spread of disease(DeMuri and Wald,2014; Oliver *et al*.,2018). Pharyngitis outbreaks are frequent in low- and middle-income nations as well as other settings where crowding is typical (such

as schools) (Carapetis *et al*.,2005) Any ages can develop GAS pharyngitis, however it is most prevalent in school-aged children, peaking at 7 to 8 years old. GAS-related pharyngitis is uncommon in children under 3 years old and significantly less frequent from late puberty through adulthood **(**Shulman *et al*.,2012 ; Chua *et al*.,2016).

Klebsiella pneumoniae is a Gram-negative bacterium that has been enclosed and can be found thriving freely in natural settings. Furthermore, *K. pneumoniae* is an opportunistic pathogen that primarily affects the lungs and urinary system, while it can also cause meningitis and sepsis in extreme situations. Multidrug-resistant *K. pneumoniae* strains, particularly carbapenem-resistant strains, are more prevalent due to widespread antibiotic use and good transmission chances have spread throughout the community and hospitals(Holt *et al*.,2015 ; Effah *et al*.,2020). *K. pneumonia* has designated as a member of the ESKAPE priority group, together with *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacter species (Founou *et al*.,2017)**.**

2-4-2 Viral causes of tonsillitis

For a number of reasons, The frequency of common pathogenic viruses in tonsillar tissue of healthy individuals has received particular attention. It has been hypothesized that the palatine tonsils are where latent infection reactivation and viral shedding take place because they serve as a reservoir for persistent viruses (like herpesviruses) in the body(Hug *et al*.,2010) Because of the Epstein-Barr virus's (EBV) well-known role in some types of lymphomas and nasopharyngeal cancer, studying the relationship between EBV and tonsillar tissue is still of interest (Shannon-Lowe and Rickinson , 2019).

Moreover, the palatine tonsils play a crucial role in immune responses to allergens and respiratory microorganisms as secondary lymphoepithelial organs. Since viral proteins are thought to have a substantial impact on the immunomodulatory responses in tonsillar tissue, the patterns of asymptomatic intratonsillar viral infections are therefore extremely important(Palomares *et al*.,2012) .There has been an increasing demand for this form of examination because tonsillar tissue is sought after as an in vivo model for studying immune responses (Jartti *et al*.,2014).

As a result of the palatine tonsils' capacity to retain viruses asymptomatically, it has also been hypothesized that there may be a connection between viral presence in tonsils and chronic adenotonsillar diseases, such as tonsillar hypertrophy and recurrent or chronic tonsillitis. This is fascinating because we still don't fully understand the causes of these widespread illnesses. Nonetheless, inconsistent findings from these evaluations have been observed. (Proenca-Modena *et al*.,2012 ; Faden *et al*.,2016).

2-5 Types of tonsillitis:

There are several types of tonsillitis, including acute and chronic tonsillitis, recurrent, as well as peritonsillar abscess.

2-5-1 Acute tonsillitis

The tonsil inflammation caused by bacteria or viruses such as double-stranded DNA viruses (human adenoviruses, Epstein Barr Virus), single-stranded DNA viruses (Human Boca Virus), single-stranded RNA viruses (influenza and Para-influenza viruses; Rhino-viruses; Entero-viruses including Coxsackie viruses; Corona viruses Respiratory

Syncytial Virus (RSV); Human meta-p (HIV). GABHS, i.e. *S.pyogenes*, are the most important microorganisms that cause bacterial tonsillitis. The illness is typically spread through droplet infection spread by other patients with acute Group A Beta Hemolytic *Streptococcus* (GABHS) tonsillitis, and very rarely by asymptomatic service providers (Kenna and Amin,2009).

Bacteria or viruses are the main causes of acute tonsillitis (Windfuhr *et* al.,2016). Laziness, constipation, overall malaise, high temperature, headache, dread of the cold, decreased appetite, and dysphagia are some of its clinical symptoms (Danstrup and Klug , 2019).

Rheumatic fever, acute nephritis, acute arthritis, myocarditis, peritonsillar abscess, parapharyngeal abscess, and other problems that have a negative impact on patients' health include acute tonsillitis' propensity to relapse(Baessler and Hale , 2019). Pediatricians and general practitioners face acute tonsillitis the most frequently in their everyday practice(Shishegar and Ashraf 2014).

The most frequent bacterial cause of acute tonsillitis is Group A - Hemolytic *Streptococcus* (GABHS), which also causes strep throat. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *chlamydia*, *pneumonia*, *pertussis*, *Fusobacterium*, *diphtheria*, *syphilis*, and *gonorrhea* are some less prevalent bacterial causes (Levinson, 2014).

Acute tonsillitis (**AT**) is characterized by the quick onset of typical clinical symptoms such as fever, general weakness, expansion of cervical lymph nodes, enlarged tonsils with possible plaque, sore throat with or without swallowing trouble, hyperaemia, and enlargement of the tonsils. These patients make up roughly 5% of all doctor visits, and 50% of them

are children between the ages of 5 and 15 .Between 70 and 95 percent of AT episodes are brought on by viral infections. In 15–30% of immune competent children and 5–15% of immune competent adults, streptococcal AT develops. Antibiotic medication is therefore not recommended in the majority of AT cases (Stelter ,2014).

The majority of acute tonsillitis treatment is supportive, with an emphasis on ensuring enough hydration, calorie intake, pain management, and temperature control. In situations of infectious mononucleosis, corticosteroids may reduce the length of the fever and pharyngitis symptoms. Gamma globulin or corticosteroids may be beneficial in cases of severe mononucleosis. Antibiotic treatment is necessary for Group A beta hemolytic Streptococci (GABHS) infection (Alasmari., 2017).

2-5-2 Chronic tonsillitis

The crypt palatine tonsil, which harbors germs and viruses, and recurrent infections are what cause chronic tonsillitis(Keskin and Guvenmez , 2019) .Clinical signs include sensations of a foreign body, pharyngeal irritation, halitosis, tonsil redness and swelling, among other things. Due to pharyngeal discomfort, patients frequently experience a bothersome cough that might interfere with daily activities (Mikola *et al*.,2018).

Since the oropharynx is an ecological system in which external and internal elements interact dynamically while maintaining an equilibrium state, the disruption of the microbiota of the tonsil mucous membranes plays a significant role in the development of chronic tonsillitis. The primary roles of an individual's indigenous microflora are detoxification, activation of hereditary and adaptive immunity, and resistance to

colonization. Any stressor that alters the microflora that typically makes up the microbiocenosis of a specific biotope might cause the immune system to lose its tolerance to the microflora and produce an immunological response (Yankovskyi,2010 ; Kamyshniy *et al*.,2011).

Chronic tonsillitis (CT) is a persistent infection brought on by frequent, recurrent infections of the tonsils. The disease's etiology results from a number of successive bouts of acute tonsillitis or from a lingering infection that causes chronic inflammation that lasts for a long time and advances slowly. All of the Waldeyer's ring's lymphatic structures are susceptible to inflammation. Inflammation most frequently affects the tonsils and adenoids in the palatine region (Plank , 2016). This illness can be categorized in a number of ways: (**a**) CT can have a bacterial, viral, or fungal etiology based on the infectious agent producing the chronic inflammation; (**b**) CT can be hypertrophic or atrophic depending on macroscopic and microscopic features; and (**c**) CT can be divided into CT in children and CT in adults depending on age. Tonsillitis is the third most common ENT (ear, nose, and throat) infection in the general population, behind rhinopharyngitis and otitis. Each year, around nine million tonsillitis cases are diagnosed in France and four million cases are reported in Spain (Haidara *et al*.,2019).

In the US, the prevalence of CT has reached 11.7%, and the number of tonsillectomies is rising. In the US, children and teenagers undergo more than 530,000 tonsillectomies annually due to chronic throat infections or sleep apnea. Children with recurrent tonsillitis have significantly worse health and physical functioning when compared to children of the same age who are healthy (Kosti´c *et al*.,2020).

2-5-3 Recurrent tonsillitis:

A low quality of life due to the disease is caused by recurrent tonsillitis. Prior to tonsillectomy, patients had an average of three attacks each year for seven years. Patients' disease-specific quality of life significantly improved after tonsillectomy. Following tonsillectomy, baseline HITP dramatically improved. The findings suggest that patients with recurrent acute tonsillitis may be subjected to unnecessary delays (Douglas, *et al*.,2017) . typically brought on by a variety of bacterial infections and recur after stopping antibiotic medication by a few weeks. Tonsillectomy may be indicated based on the frequency and severity of such episodes (Stelter, 2014)**.**

In inflammatory illnesses, lymphocytes are crucial for cytokine generation in the chronic process, but neutrophils are necessary for the acute process (Wang and Arase, 2014).

Moreover, increased neutrophil counts show an acute inflammation, whereas lower lymphocyte counts show a body lacking in some nutrients and a persistent inflammatory state. The parameter of systemic inflammation proposed is NLR(Achar *et al*., 2015). NLR is a useful measure in conditions including sudden hearing loss, certain types of cancer, autoimmune and cardiovascular illnesses, and it may be easily detected by a straightforward complete blood count analysis(Patel *et al*.,2014., Chung *et al*.,2015).

2-5-4 Peritonsillar abscess:

Are clumps of purulent material that typically form close to the superior pole between the pharyngeal constrictor muscles and the tonsil fibrous capsule(Papacharalampous *et al*., 2011; Blair *et al*., 2015).

From acute tonsillitis to peritonsillar tonsillitis, the PTA is likely an evolution (Papacharalampous *et al*.,2011). According to some authors, crypt blockage in acute tonsillitis causes drainage failure of suppurative inflammation, which causes the infection to expand into the peritonsillar space. Others argue that Weber's glands, or salivary glands, in the supratonsillar region, may get infected as a result of PTA development (Blair *et al*.,2015).

2-6 Immunity of tonsillitis

The palatine tonsils appear to be an immune system component that protects against upper respiratory tract infections(Aljurayyan *et al*.,2018) .Tonsil ring lymphoid tissue, also known as mucosa-associated lymphoid tissue (MALT), is found in the upper respiratory tract and is comparable to other lymph epithelial tissues mass in the lining and intestinal system(Ahn *et al*.,2018). This tissue, like other lymphoid organs, has the capacity to mount targeted immune responses in response to diverse antigens (Vistarop *et al*.,2018) .This potential is obvious, and the immunological interactions of the environment, particularly throughout childhood, lead to the hyperplasia of the palatine tonsils(Brambilla *et al*.,2014).

The lymphatic tissue of the tonsils plays a smaller role in the immune system as it matures, which lasts until 8 to 10 years of ageThe amount of lymphocytes in each region of the tonsils also declines during this time(Els ;Olwoch,2018). Although the immunological relevance of the tonsils declines with age, the tonsil tissues continue to function as immune cells even as they get older, thus the decision to have a tonsillectomy if there is a valid rationale will not be impacted (Mabbott *et al*.,2015). A severe tonsillar hyperplasia (enlarged tonsils) and

considerable functional stenosis in the bottleneck may result from childhood tonsillar immunity, which can restrict breathing and create difficulties eating (Subramanyam *et al*.,2013).

Children may endure a severe respiratory disorder with apneic episodes, especially when they are sleeping. Also, these kids are exposed over time to a higher risk of developing cor pulmonale (Kasle *et al*.,2016 ; Cayer , 2017) .The immunologic profile of patients before and after tonsillectomy is crucial information for otolaryngologists and primary care doctors to be aware of, especially in youngsters. The tonsillectomy is generally not advised, especially in youngsters. Only a small number of research have been done on the long-term consequences of tonsillectomy on patients' immune systems. Many studies have been done on the short-term effects of tonsillectomy on the immune system (Damiani *et al*.,2016 ; Kawasaki et al.,2017).

By activating CD4+ T cells, which have T cell antigen receptors (TCR) that identify short peptides attached to MHC-II molecules (pMHCII) generated by host antigen-presenting cells, GAS can survive in lymphoid tissue and cause a humoral immune response. Then, these T cells are encouraged to proliferate and differentiate into Th1, Th2, or Th17 effector cells through the production of cytokines like IL-6, IL-10, or IL-17 (Dileepan *et al*.,2011).

Immune cells, mostly lymphocytes, are present in every compartment of the tonsils, including the lymphoepithelium, the inter-follicular gaps, and the follicles. Instead of CD8+ T cells, CD4+ T cells make up the majority of the intraepithelial lymphocytes, and 50% of them are B cells. Similar to this, B cells dominate secondary lymphoid follicles, where they go through strong maturation and rapid

differentiation. The germinal center also contains follicular T cells and follicular DC in addition to B cells. Interestingly, shown that each of the five tonsillar T cell developmental stages mimics its thymic counterpart. This research suggests that the tonsils of humans may undergo the whole range of T cell development stags (McClory *et al*.,2012) .The ability of the tonsils to serve as the first-line organ in the establishment of tolerance has also been shown by the creation of Foxp3+ T regulatory cells that are specific to allergens. It has been shown that PT has more CD4+Foxp3+ Treg cells specific for Bet v 1 than peripheral blood (Palomares *et al*.,2012) .In addition to tolerance induction, When TLR4, TLR8, and pro-inflammatory cytokines are triggered in the tonsil, allergen-specific T cell tolerance may also be negatively impacted(Kücüksezer *et al*.,2013).

2-6-1 Adaptive immune response

Due to their potential to differentiate into cells that produce antibodies specific to an antigen after exposure to microbes or vaccination, B lymphocytes are essential components of the immune system's reactivity. A few of the B cell subpopulations that have been discovered through their distinctive phenotypic arrays of surface markers include memory, germinal center, and follicular B cells. Collectively, these populations include conventional B cells (sometimes referred to as B-2 B cells), which undergo affinity maturation to develop into plasma cells and respond nimbly to antigen challenges with antibody responses (Baumgarth ,2011). Several populations of B cells have been identified and categorized as innate immune system components in recent years (Rothstein *et al* , 2013). B-1 B cells, which constitutively and spontaneously release "natural" antibodies required as the first line of defense against infections, and marginal zone (MZ) B cells, which are specialized in responses to blood-borne illnesses (Martin *et al* 2001).

And B-10 B cells, which produce IL-10 and possess immunosuppressive qualities (Horikawa *et al*.,2013).

Immunologists have been researching lymphocyte antigen recognition ever since antibodies and their specificities were discovered more than a century ago (Kaufmann , 2017)., The 1960s–1980s saw the more recent discovery of T lymphocytes and their antigen receptors (Miller , 2002). In lymphoid organs' germinal centers (GCs) and extrafollicular areas, B cells that produce neutralizing antibodies are produced(Victora,& Nussenzweig,2012; DeSilva & Klein, 2015). Upon antigen presentation by antigen-presenting cells (APCs) (Gerner *et al*.,2017).

T follicular helper (TFH) cells interact with a number of hematopoietic and non-hematopoietic cells to send signals to GC B cells for differentiation, survival, proliferation, maturation of antibodies, and affinity maturation (Crotty , 2014). In vivo experiments using inbred mice have been used to clarify almost all of these connections. Despite the fact that they have generated a wealth of useful information (Cyster and & Allen , 2019 ; Qi *et al*.,2014) .Several mechanistic aspects are unavailable due to the unavailability of a system that replicates the crucial aspects of human adaptive immunity, such as affinity maturation and class switching, and the impacts of adjuvants. This is crucial for vaccine testing since many vaccine candidates that were successful in animal models eventually fall short in human trials (Jameson, & Masopust , 2018).Peripheral lymphoid organs like the tonsils, lymph nodes, and spleen generate GCs during an adaptive immune response. B cells gather in the GC region, while T cells are mostly found in peripheral locations (Qi *et al*.,2014).

2-6-2 Innate immune response

Innate immune cells use a variety of receptor families, Group A *Streptococcus*, often known as *Streptococcus pyogenes*, is a significant Gram-positive human pathogen, however it is unknown if innate immune cells can recognize it (Tsatsaronis and walker.,2014., Fieber and Kovarik , 2014). A wide variety of typically self-limiting illnesses, including as pharyngitis (strep throat), scarlet fever, or impetigo, are brought on by *S. pyogenes* (Johansson *et al*.,2010 ; Wessels , 2011) .

Moreover, it has the potential to lead to invasive, fatal diseases including toxic shock and necrotizing fasciitis. Around 700 million mild and over 650,000 severe invasive infections caused by *S. pyogenes* occur each year throughout the world.(Carapetis *etal*.,2005) One of the co-infecting bacteria most frequently discovered in samples from the 1918 flu pandemics and in patients from the most recent H1N1 flu outbreak, along with *S. pneumoniae*, is *S. pyogenes*. (Morens and Fauci, 2007)

The TLRs and PAMPs involved in the functional recognition of *S. pyogenes* have not been discovered, despite the significance of the innate immune system for host defense. Both conventional dendritic cells (cDCs; bone marrow cells differentiated in the presence of GM-CSF) and *S. pyogenes*-induced murine bone marrow-derived macrophages (BMDMs; bone marrow cells differentiated in the presence of CSF1) have been shown to require the signaling adaptor MyD88. When infected with *S. pyogenes*, MyD88 is continuously needed for mouse survival. (Loof *et al*.,2010) Unknown TLRs are responsible for the protective innate immune response. Research conducted by us and others have shown that *S. pyogenes* can cause the production of cytokines even in the

absence of the MyD88-dependent TLR2, TLR4, and TLR9 (Gratz *et al*.,2008).

2-7 Mucosal immunity

The lymphoid epithelial tissues of the oral mucosa surrounding the oropharynx are known as human paired palatine tonsils. Together with the adenoids and lingual tonsils, they are a component of the Waldeyer's ring of lymphoid tissue. Because to their continual exposure to dietary and airborne antigens, the palatine tonsils (referred to as tonsils from now on) are placed in a way that promotes the development of mucosal immunity (Ags). Moreover, they have developed without Ag-degrading digesting enzymes to directly carry foreign material from the exterior to the lymphoid cells through deep and branched crypts (Brandtzaeg , 2003).

Many microorganisms, primarily bacteria, colonize the human body's surfaces, including the oropharynx, and develop a mutualistic connection with the host. Tonsils are mostly B-cell organs and reach their peak immunological activity between the ages of 4 and 10. For reasons that are still unknown, certain children and adults (who were not included in this study) have hyperplasia and hypertrophy of the tonsils. The primary pathophysiological symptom underlying OSA is such enlargement., a serious public health concern that is extremely common. Repeated episodes of partial or total upper airway obstruction during sleep that prevent normal breathing and all the problems that follow owing to hypoxemia are the hallmarks of OSA. Blood from OSA patients has been found to have an increase in pro-inflammatory cytokines. (Kheirandish-Gozal and , Gozal , 2019)

Throughout inflammatory, autoimmune, and viral disorders, B cells support immunological responses. The last two decades have shown us

that B cells can control physiological and pathological processes not only by generating antibodies (Abs) and presenting Ags, but also by creating cytokines. It has been demonstrated that B cells can exist as a variety of cytokine-secreting subsets with pro- or anti-inflammatory properties. (Fillatreau , 2018).

The tonsils provide a platform for studying such subsets since they are a significant source of human B lymphocytes. Within this context, we recently showed that tonsils removed due to recurrent tonsillitis produced significantly more interleukin 10 (IL10) producing B cells (Bregs) than OSA tonsils, demonstrating that Bregs play a more complex and intriguing role in tonsillar disease than was previously recognized. Moreover, this Breg population flaw was associated with a rise in the percentage of germinal center (GC) cells. Because that tonsils are hypertrophied by GC cells, this association suggests that the Breg subgroup regulates GC responses(Sarmiento Varon *et al*.,2017).

2-8 Cytokines

Cytokines are chemical mediators that are released by many different types of cells in the human body. They allow cells to communicate with one another. They function as chemical messengers both within the immune system and between the immune system and other body systems. a cell can detect the presence of a cytokine if it has surface receptors that recognize the cytokine. The cytokine causes (mediates) a response in a cell that detects its presence. Some cytokines act as chemo attractants, attracting phagocytes to areas where they are needed. Others, such as interferon's, play a direct role in host defense (Sell and Edward, 2001).

The cytokines include a large number low-molecular weight glycoproteins (lower than 80 kD) that act in cell intercommunication,

They can be secreted and/or expressed in cell membranes of the extracellular matrix , that participate in modulating inflammatory and immune reactions in many diseases, that influence the function of every organ system (Wine and Alper, 2012).

2-9 Cytokines and the role of CD4,CD19 , IL3and IL9

Intercellular communication mediated by cytokines is the primary mechanism by which immune system cells communicate with one another. Many aspects of cytokine signaling in the immune system have been thoroughly investigated at the structural, biophysical, biochemical, and cellular levels (Altan-Bonnet and Mukherjee, 2019).

2-9-1 CD4 and tonsillitis

Key participants in the adaptive immune response are CD4+ T cells. Naive CD4+ T lymphocytes travel to the periphery after being selected and matured in the thymus, where they look for antigens that are expressed by HLA-II molecules on the surface of skilled antigen-presenting cells (APCs)(Rossjohn *et al*.,2015) .Such as Dendritic cells (DCs) (Ohno *et al*.,2016) B cells (Rijvers *et al*.,2020) macrophages (Codolo *et al*.,2020) CD4+ T cells and airway and intestinal epithelial cells (Wosen *et al*.,2018) **.**

Circulating memory Tfh (cTfh) cells are thought to represent populations of CD4+ memory T cells in the blood that exhibit characteristics similar to lymphoid Tfh cells(Crotty, 2018). Although a limited number of circulating PD-1hiCXCR5hi CD4+ T cells are also present, Tfh GC cells express CXCR5, PD-1, and ICOS at far higher levels than peripheral cTfh cells do (Vinuesa *et al*., 2016). Despite disagreement over the phenotypic description of circulating Tfh cells, it is

agreed that these cells promote immunoglobulin (Ig) class switching and plasma blast formation in co-culture with naive or memory B cells(Locci *et al*., 2016). Based on similarities to classical Th CD4+ cell subpopulations, three distinct subsets of cTfh cells have been identified: Th1-like (CXCR3+CCR6), Th2-like (CXCR3CCR6), and Th17-like (CXCR3CCR6+) cTfh cells. The diversity of cTfh cells is further demonstrated by the differences in cytokine production and transcription factor expression that are observed when various cTfh cell subsets are co-cultured with naive B cells in the presence of staphylococcal enterotoxin B (SEB). Interferon (IFN-), IL-4, IL-5, and IL-13 are all produced by Th1-like subsets. Th17-like subsets also produce IL-17A and IL-22. (Bentebibel *et al*., 2013).

The T cell receptor (TCR) is a fixed marker of the clono type. Adult volunteers having tonsillectomy provided donor-matched tonsils and peripheral blood in order to evaluate the TCR repertoires of Tfh and non-Tfh cell subsets. However, there was no distinction between them and CXCR5 memory CD4+ T cells between tonsillar and peripheral Tfh cells. The interaction of blood memory Tfh and non-Tfh cells with the hemagglutinin (HA) protein of the influenza virus was also studied. (Fazilleau *et al*., 2007; Sant *et al*., 2018).

2-9-2 CD19 and tonsillitis

Recurrent bacterial and viral infections are the primary underlying causes of tonsillitis, which is frequently a self-limiting localized inflammation of the oropharynx. Because these disorders have the potential to alter the tonsils' histomorphology and function as a result of continuing antigenic stimulation, tonsillectomy is necessary (Gysin,2013). The host's defense against invaders of the upper respiratory

tract is greatly aided by the tonsils, which are the location of intraepithelial immunoglobulin-producing B cells that account for around 50% of the total cell population (Van Kempen *et al*.,2000). T cells make up a smaller portion of tonsillar lymphocytes and have more CD4 than CD8 cells; the former are typically found in groups with B cells. About 15% of B cells are B1 lymphocytes in the tonsil sub epithelial region. In fetal life, the B1a subpopulation of B1 cells, which are distinguished by their surface co-expression of CD5 and CD19, predominates, and their frequency declines with maturity. It's interesting to note that older people may once again experience an increase in B1a cell frequency The lymph nodes, spleen, extra nodal tissue, and tonsils all contain B1a cells. B1a cells' primary function is to create "natural," low-affinity, and poly reactive IgM, which can bind a variety of bacterial antigens and auto antigens, serving as a link between innate and adaptive immune responses. (LeBien and Tedder,2008).

2-9-3 IL9

The Th9 subgroup of T cells that express interleukin (IL)-9 improves immunity against helminthic parasites and exhibits anticancer activity(Kaplan *et al*.,2015; Licona-Limon *et al*.,2013).

Additionally, Th9 cells support allergic inflammation and autoimmune illness(Kaplan *et al*.,2015) .Several transcription factors, including STAT5, STAT6, nuclear factor-B (NF-B), NFAT, PU.1, IRF4, IRF8, Foxo1, and BATF, stimulate the production of IL-9, albeit none of them are known to be lineage-determining factors(Kaplan ,2017; Bi ,2017). It was discovered that the super-enhancers surrounding the *Il9 gene* intersect with conserved regulatory regions(Xiao, *et al*,2018 ; Schwartz *et al*.,2019). and play a crucial role in the efficient production of IL9 by Th9

cells. Other Th lineages have been shown to express IL9 at a lower level. Tumor necrosis factor superfamily members and PU.1 have the ability to boost the expression of IL-9 in regulatory T cells (Tregs) and Th17 cells, respectively (Kim *et al* , 2015; Xiao *et al*,2015).

It is common in tonsillitis, causes the production of Th1 cytokines like IFN- and TNF, and during the release of Th2 type cytokines, tissues have elevated levels of TNF-, IL-1, IL-6, IL-9, and IL-13. Due to the activation of single-celled macrophages brought on by repeated stimulation by pathogenic substances, which results in an excess of inflammatory cytokines (Mikola,*et al* 2018).

Infectious and allergic disorders. It has been proposed that IL-9 plays a crucial role in inflammatory reactions of the airway based on the findings that mast cells and Th2 cells release IL-9 during inflammatory responses (Gounni *et al*.,2000). For this reason, most studies on *IL-9* genes polymorphisms focus on allergy and infectious respiratory tract illnesses. Additionally, IL-9 polymorphisms are related to disparities between the sexes: Girls are more susceptible to developing severe respiratory syncytial virus infections when carrying the *IL-9 gene* variant *rs2069885*. (Schuurhof *et al*.,2010).

Specific sex associations of *IL-9* rs2069885 were also demonstrated with asthma in males (Aschard *et al*.,2009) .These findings suggest that differences in immune-related genes, due to SNPs, have a nonidentical effect on the severity of disease, and indicate the importance of heterogeneity according to sex and pleiotropy (Schuurhof *et al*.,2010 ; Aschard *et al*.,2009).

Significant *IL-9 rs1859430, rs11741137*, and *rs2069885* dominant genotype manifestations in asthma individuals showed that these patients

were more prone to experience a severe asthma exacerbation brought on by increased dust mite exposure(Sordillo *et al*.,2015). Additionally, the *IL9R/IL9* producing genes' *rs731476 T-/rs2069885* G-genotype combination has been linked to a considerably increased incidence of allergic rhinitis in women(Fatahi *et al*.,2016).

Various illnesses. Graves' disease and Graves' ophthalmopathy may both have *rs1859430* polymorphisms as a co genetic risk factor, according to research on *IL-9* polymorphisms in autoimmune illnesses. (Zhu *et al*.,2010). have hypothesized that *IL-9 rs2069885* may be associated with an increased risk for active migraine without aura (Schürks *et al*.,2009).

2-9-4 IL3

IL-3 is a member of the hematopoietic cytokine family with four short -helices, along with GM-CSF and IL-5. All three cytokines attach to distinct -receptor subunits, but they all employ the same -receptor subunit to transmit signals, mostly through the JAK/STAT pathway (Broughton.,2012). but can also be expressed by microglial cells, basophils, neurons, and innate response activator B cells (Weber *et al*., 2015)basophils, neurons, and microglial cells (Schroeder *et al*.,2009). According to(Didichenko *et al*. 2008), IL-3 causes a variety of target cells, such as mast cells, basophils, monocytes, DCs, B cells, T cells, and endothelial cells, to become activated or to live longer. In a model of sepsis (Weber., 2015). As well as in models of arthritis and lupus nephritis .A significant role for IL-3 in inflammation and autoimmunity has recently been demonstrated. Monocytes and BM cells are activated by IL-3 to generate pro inflammatory cytokines, and it also has ant

apoptotic effects on a variety of leukocytes. Endothelial cells are also activated by IL-3 to upregulate E- and P-selectin (Renner .,2015).

2-10 Gene polymorphism

Over 80 million autosomal single nucleotide polymorphisms (SNPs), including 8 million common variants, have been discovered in genomic surveys of individuals from multiple populations (Auton *et al*., 2015)

Genome-wide association studies (GWAS) have identified disease risk associations for thousands of these SNPs (Visscher *et al*., 2012).

However, because the vast majority of GWAS hits occur in non-coding regions of the genome, little is known about which genes they affect, which cell types they act on simultaneously, large-scale epigenomic initiatives examining multiple human cell types have assigned cis regulatory function to nearly 20% of non-coding DNA sequences and demonstrated that disease risk variants are enriched in cell-specific cis regulatory DNA sequences (Kundaje *et al*., 2015).

This suggests that disease risk variants are likely to influence gene expression in a context-dependent manner in a subset of human cell types. The identification of the cell types in which disease risk variants modulate gene expression will aid mechanistic and functional studies into the genetic basis of specific diseases. The Genotype-Tissue Expression (GTEx) consortium investigated the relationship between genetic variants and gene expression levels (expression quantitative trait loci, or eQTLs) in 44 human tissues (Battle *et al*., 2017).

Its findings are useful for narrowing down tissue-specific cis regulatory effects of disease risk variants, but they do not include information on eQTLs in immune cell types. Previous large-scale eQTL

studies of human immune system cells were conducted in a variety of cell types and cell lines (Zhernakova *et al*., 2017).

2-11 Single nucleotide polymorphisms (SNPs)

SNPs, or single nucleotide polymorphisms (pronounced "snips"), are the most common type of genetic variation in humans. Each SNP represents a difference in a single DNA building block known as a nucleotide. For example, in a specific stretch of DNA, an SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T). SNPs occur naturally throughout a person's DNA. These variations are most frequently found in the DNA between genes. They can function as biological markers, assisting scientists in identifying genes linked to disease (Srinivasan *et al*., 2016).

Depending on their genomic location, mutations have the potential to change all stages of gene expression. They can influence mRNA expression when found in transcriptional regulatory elements. SNPs can affect mRNA splicing, nucleocytoplasmic export, stability, and translation when they occur in genes. When they occur within a coding sequence and result in an amino acid change (referred to as a non-synonymous SNP or mutation), they can alter the protein's activity. If the mutation is synonymous (i.e., does not change the nature of the amino acid), translation rates or mRNA half-life may be affected. If the mutation results in a premature stop codon, this can result in the production of a truncated protein product or a near-null phenotype due to nonsense-mediated decay (Mendell and Dietz, 2001; Nicholson *et al*., 2010).

Cytokine genes are highly conserved, especially in the promoter and coding regions; any non-conservative mutations within these regions may affect cytokine production. High levels of transcription, production, and

functional activity of inflammatory or anti-inflammatory cytokines can sometimes be attributed to genetic variations such as single nucleotide polymorphisms (SNPs), altered coding regions, and promoter or regulatory regions of cytokines genes. Furthermore, SNPs in certain pro-inflammatory cytokine alleles resulted in susceptibility to a variety of infections (Zhao *et al*.,2017; Mohsen *et al*.,2019). However, some of these genetic differences have proven to be extremely important in the study of human health. SNPs discovered by researchers may help predict an individual's response to specific drugs, susceptibility to environmental factors such as toxins, and risk of developing specific diseases. SNPs can also be used to track disease gene inheritance within families (Green and Guyer, 2011).

2-12 Polymerase chain reaction (PCR):

Polymerase chain reaction (PCR) refers to a technique employed widely in the basic and biomedical sciences. PCR is a laboratory technique utilized to amplify specific segments of DNA for a wide range of laboratory and/or clinical applications. Building on the work of Panet and Khorana’s successful amplification of DNA in-vitro, Mullis and coworkers developed PCR in the early 1980s, having been met with a Nobel prize only a decade later. Allowing for more than the billion-fold amplification of specific target regions, it has become instrumental in many applications including the cloning of genes, the diagnosis of infectious diseases, and the screening of prenatal infants for deleterious genetic abnormalities, The main components of PCR are a template, primers, free nucleotide bases, and the DNA polymerase enzyme. The DNA template contains the specific region that you wish to amplify, such as the DNA extracted from a piece of hair for example. Primers, or oligonucleotides, are short strands of DNA complementary to the 3' end

of each target region. Both a forward and a reverse primer are required, one for each complementary strand of DNA. DNA polymerase is the enzyme that carries out DNA replication. Thermo stable analogues of DNA polymerase I, such as Taq polymerase, which was originally found in a bacterium that grows in hot springs, is a common choice due to its resistance to the heating and cooling cycles necessary for PCR (Mullis, 1990).

PCR takes advantage of the complementary base pairing, double-stranded nature, and melting temperature of DNA molecules. This process involves cycling through 3 sequential rounds of temperature dependent reactions: DNA melting (denaturation), annealing and enzyme-driven DNA replication (elongation). Denaturation begins by heating the reaction to about 95 C, disrupting the hydrogen bonds that hold the two strands of template DNA together. Next, the reaction is reduced to around 50 to 65 C, depending on the physicochemical variables of the primers, enabling annealing of complementary base pairs (Wittwer *et al*., 2013).

The primers, which are added to the solution in excess, bind to the beginning of the 3' end of each template strand and prevent re-hybridization of the template strand with itself. Lastly, enzyme-driven DNA replication begins by setting the reaction temperature to the amount which optimizes the activity of DNA polymerase, which is around 75 to 80 C. At this point, DNA polymerase, which needs double-stranded DNA to begin replication, synthesizes a new DNA strand by assembling free-nucleotides in solution in the 3' to 5' direction to produce 2 full sets of complementary strands. The newly synthesized DNA is now identical to the template strand and will be used as such in the progressive PCR cycles. Given that previously synthesized DNA strands serve as templates, the amplification of DNA using PCR increases at an

exponential rate, where the copies of DNA double at the end of each replication step. The exponential replication of the target DNA eventually plateaus around 30 to 40 cycles mainly due to reagent limitation, but can also be due to inhibitors of the polymerase reaction found in the sample self-annealing of the accumulating product, and accumulation of pyrophosphate molecules (Ishmael and Stellato, 2008).

2-13 Allele-specific PCR or The amplification refractory mutation system (ARMS):

A diagnostic or cloning technique based on single-nucleotide variations (SNVs not to be confused with SNPs) (single-base differences in a patient). Any mutation involving single base change can be detected by this system. It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNV (base pair buffer around SNV usually incorporated). PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP or small deletions in a sequence See SNP genotyping for more information (Bulduk., 2022).

A tetra-primer ARMS-PCR method to detect a single leptin mutation using genomic DNA collected from mice's tails.Tetra-primer ARMS-PCR has been applied to identify the single nucleotide polymorphism (SNP) (Islam *et al*., 2021) and differentiate whether the single mutation in DNA is heterozygous or homozygous (Komijani *et al*., 2022).

This method takes advantage of the relative inability of Taq DNA polymerase to extend primers mismatched at their 3′-end and its intrinsic

propensity of lacking 3′ to 5′ exonuclease activity, so the extension is dramatically reduced (Vamvakopoulos, 2002).

In the tetra-primer ARMS-PCR assay, the outer primers are non-allele-specific and used to amplify the region that comprises the SNP For inner primers, the last nucleotide at the 3′ end of the primer is designed to be complementary to the target nucleotide. An additional mismatch within the three bases closest to the SNP site at the 3′ end of the inner (Medrano and de Oliveira 2014).

Chapter Three

Materials and Methods

3-1 Materials

3-1-1 Instrument and apparatus

Instrument and apparatus that used in this study are illustrated in Table(3-1)

Table (3-1) Instruments and apparatus

Instruments and apparatus	Company	Country origin
Autoclave	Tripod	UK
Beakers and flasks	Steriline	UK
Bench centrifuge	Memmert	Germany
Burner	Amal	Turkey
Different size of tips	Meheco	China
Different size of tips	Meheco	China
Digital camera	Sony	Japan
Electric sensitive balance	Denver	USA
ELISA reader	Biotech	USA
Eppendorf tubes	Eppendorf	Germany
Gel documentation system	Vilber	France
Gel electrophoresis system	Cleaver Scientific	UK
High speed centrifuge	Hettich	Germany
Hood	Bio LAB	Korea
Horizontal electrophoresis unit	Cleaver	UK
Incubator	Selecta	Spain
Light microscope	Olympus	Japan
Loop	Shndon	UK
Microcenterfuge tubes	Biobasic	Canada
Micropipettes	Capp	Denmark)
Oven	Olympus	Japan
PCR Thermal cycler	Techne	UK
Petri dishs	Sterilin	England
Plain tubes	DMD-DISPO	Syria
Refrigerator	Kiriazi	Egypt
Slides	Sail Brand	China

Sterile hypodermic syringe	EL-dawlia ico	Egypt
Sterilize swab	ATACO	Brand
UV transilluminator	ATTA	Korea
Vitek 2 system	Biomerieux	France
Vortex mixer	Griffin	Germany

3-1-2 Chemical and biological materials:

Chemical materials, reagents, stains and solutions used in the present study illustrated in Table (3-2)

Table (3-2): Chemical and biological materials

Type of chemical-Biological material	Company/origin
6X DNA Loading buffer Blue	Eurx- Poland
Agarose	Condalab- spain
Ethanol 99%	Merck-England
Glycerol ($C_3H_8O_3$)	Merck-England
Gram stain set	BDH- England
Ladder	Bioneer- korea
Nuclease free water	Bioneer - india
Phosphate buffer saline PBS	Sigma (USA)
Primers	Bioneer- korea
Proteinase K	Bio basic-Canada
Red safe dye	Bio basic⁄ Canada
TBE buffer 10 x	Intron – korea

3-1-3 Culture media

According to Forbes *et al*. (2007) and McFadden (2000) main culture media used in this study are listed in Table (3-3) . All media were prepared according to the manufacturer's (Hi-media (India)) and 5% fresh human blood was added to blood agar base after sterilization to prepare blood agar.

Table (3-3): Culture media

Type of media	Manufacturing company	Origin
Blood agar base	Himedia	India
Brain heart infusion broth	Himedia	India
Chocolate agar	Himedia	India
MacConkey agar	Himedia	India

3-1-4 Commercial kits

Commercial kits were used in the present study are illustrated in Table (3-4).

Table (3-4): Commercial kits

Type of Kit	Company / Country
DNA extraction Kit	Favorgen(Taiwan)
Human CD4 , CD19, IL3 and IL9 (ELISA)	BT lab /China
DNA ladder 100bp	Bioneer/ Korea
Vitek 2 system kit	Biomerieux(france)

3-1-5: Content of ELISA kits

Content of ELISA kits are shown in Table (3-5)

Table (3-5) The content of ELISA kits

Components	Quantity
Biotinylated Human FCN2 Antibody	1ml*1
Plate Sealer	2 pics
Pre-coated ELISA plate	12*8 well strips *1
Standard Diluent	3ml*1

Standard solution (32ng/ml)	0.5ml*1
Stop solution	6ml*1
Streptavidin- HRP	6ml*1
Substrate Solution A	6ml*1
Substrate Solution B	6ml*1
User Instruction	1
Wash Buffer Concentrate (25x)	20ml*1
Zipper bag	1 pics

3-1-6 DNA extraction kits for human RBCs (FAVROGEN) .

Human genomic DNA was extracted from frozen white blood cells (WBCs) by using Favorgen kit Table (3-6).

Table (3-6) DNA extraction kit for frozen blood

Cat . No. / Preps	FABGK 100 (100 PREPS)	FABGK 300 (300 PREPS)
2 ml collection tube	200 pcs1	100 pcs
Elution buffer	30 ml	75ml
FABG Buffer	40 ml	100 ml
FABG column	100 pcs	300 pcs
FATG Buffer	30 ml	75 ml
RBC lysis Buffer	135 ml	405 ml
W1 Buffer	45 ml	130 ml
Wash Buffer concentrated	25 ml	50 ml

3-1-7 Sequences of Primers and DNA marker used specific allele of IL3 and IL9 gene

Primers that used in the current study were listed in the Table (3-7)

Table (3-7) Primers for amplification of IL3 and IL9

Primers	Sequence (5'-3')		Size of product
IL-3 **polymorphism "rs40401"**	**F**	Forward primer 5-CAGGCGTCGGAAGGATCTTTATC-3	**337 bp** **(Current study)**
	R 1	Reverse primer C allele 5-CAATTGGGTCGAACAGAAGTTACC-3	
	R 2	Reverse Primer T allele 5-CAATTGGGTCGAACAGAAGTTCCT-3	
IL9 **polymorphism (rs2069870)**	**F1**	Forward A allele 5-CTTTACATGCATTAAGCCATGTAATACA-3	**333bp (Current study)**
	F2	Forward G allele 5-CTTTACATGCATTAAGCCATGTAATACG-3	
	R	**Reverse 5-CAGGAGTTCAGGAGGATTAAGAGCT-3**	

3-1-8 Master mix used in PCR

The master mix used in PCR that were used in this study are shown in Table (3-9)

Table (3-8): Master mix used in PCR

Master mix 2x
Taq DNA polymerase, 250 µm dNTP (dATP, dGTP, dCTP, TTP), 1.5mM $MgCl_2$, 30mM KCl, 10mM Tris-HCl (pH 8.3), tracking dye

3-1-9 DNA ladder

The DNA ladder that were used in this study are shown in Table (3-9)

Table (3-9) DNA ladder

Materials
1-A ladder consists of 13 double –stranded DNA with size 100-2000 bp 2-Loading dye has a composition (15% Ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orangeG, 10 Mm.tris-HCl, pH 7.5 and 50 mM EDTA).

3-1-10 Reaction mixture of PCR

The contents of the reaction mixture of PCR that were used in this study are shown in Table (3-10).

Table (3-10): Contents of the reaction mixture of PCR

NO.	Contents of reaction mixture	Volume
1	Green master mix	12.5 µl
2	Upstream primer	1µl
3	Downstream primer	1µl
4	DNA template	3µl
5	Nuclease free water	7.5µl
Total volume		25 µl

3-2 Methods

3-2-1 Media and biological materials preparation of culture media

The culture media were prepared according to the manufacture company and sterilized by autoclave at 121ċ,1Bar(14.5psi) and 15 min (Macfadden, 2000).

3-2-1-1 MacConkey agar medium

It was used for the primary isolation of most Gram-negative bacteria and to differentiate lactose fermenters from non-lactose fermenters (Winn *et al*., 2006).

3-2-1-2 Chocolate agar

It used for primary culturing of the bacterial special *Streptococcus* (Forbes *et al*.,2007).

3-2-1-3 Blood agar medium

Blood agar medium was prepared according to manufacturer instructions by dissolving 40 g of blood agar base in 1000 ml D.W. The medium was autoclaved at 121 ºC for 15 min and pressure 15 square (psi), cooled to 50 ºC and 5% of fresh human blood was added. This medium was used as enrichment medium for the cultivation of the bacterial isolates and to determine their ability of blood hemolysis (Forbes *et al*., 2007)

3-2-1-4 Brain heart infusion broth with 15% glycerol

This medium was prepared by adding 5 ml of glycerol to 95 ml of BHI broth before autoclaving at 121 ºC for 15 min and pressure 15 square (psi). The medium was used in preservation of bacteria (MacFadden, 2000).

3-2-1-5 Gram stain solution

Gram stain solution was supplied from BDH-England . The solution was used to study Gram positive and Gram negative bacterial cells ,morphology and their arrangement (Forbes *et al.*, 2007).

3-3 Preparation of molecular materials

3-3-1 Tris borate EDTA (TBE) buffer (bio-basic / England)

Prepared 500 ml of TBE (1X) by adding 50 ml of TBE (10X) stock solution to a final volume of 500 ml of distilled water (d H_2O) . Prepared 500 ml of TBE (0.5X) by adding 50 ml of TBE (10X) stock solution to a final volume of 500 ml of d H_2O .

3-3-2 Preparation of agarose gel

This gel was prepared by adding (1.5 agarose in 100 ml TBE buffer) to be dissolved by boiling, then it was left to cool to 50ºC. The dissolved amount of agarose powder is depending upon the aim for which agarose was used for visualization of PCR product.

3-3-3 Rehydration of primers

Lyophilized primer pairs were rehydrated by DNA rehydration solution 1X (pH 8.0) Tris- EDTA buffer (TE-buffer). Initially, primer storage-stock tube preparedand then the working solution would prepared from primer stock tube. Consistent with the instructions of the producer (Bioneer/Korea), TE buffer was added to produce 100 picomole/microliter concentration of primer stock solution. The working solution prepared from stock as 1:10 (v/v) by dilution with TE buffer to get 10 picomole/microliter at 4°C.

3-4 Study population

3-4-1 Specimens collection

The current study included a comparison of cellular immune parameters that occurs at the level of systemic immunity and local immunity for patients with acute ,chronic and recurrent tonsillitis caused by Gram-positive and Gram-negative bacteria for the period from March to September 2022. The study included 135 specimen 90 patients and 45 control , the patient including 51 males and 39 females who attended Imam Hassan Hospital Al-Mujtaba and some outpatient clinics in Karbala suffer from tonsillitis. They were diagnosed by an otolaryngologist and 45 people of the same age were considered as a control group. The number of males was 28 and females were 17. For all patients and healthy people, the blood samples and swab smears were taken from infected area by sterilized cotton swab, and then samples had been inoculated on brain heart infusion broth and incubate at 37C for 24 hrs. and then on different bacterial culture media (MacConkey, Blood and Chocolate agar) incubated aerobically and anaerobically at 37 ^{o}C for 24-48 hrs.

The whole blood sample was collected from each participant, noting that the sera were used to determine CD4,CD19,IL3,IL9concentration by ELISA, while EDTA blood was used to DNA extract. Five milliliters of venous blood were obtained from each subject , one milliliter was put into EDTA tubes and the remaining four milliliter pushed slowly into disposable tubes containing separating gel . Blood in the EDTA tubes was stored in -20^{o}c in order to be used later in genetic study , while blood in the gel containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000 x g for approximately 15 minutes

then the serum were obtained and stored at -20ºc until used.DNA extraction was used to detect SNPS in IL3 and IL9 by used (specific allele).

3-5 Study Scheme

The specimens were proceed according to study design that showed in Figure (3-1).

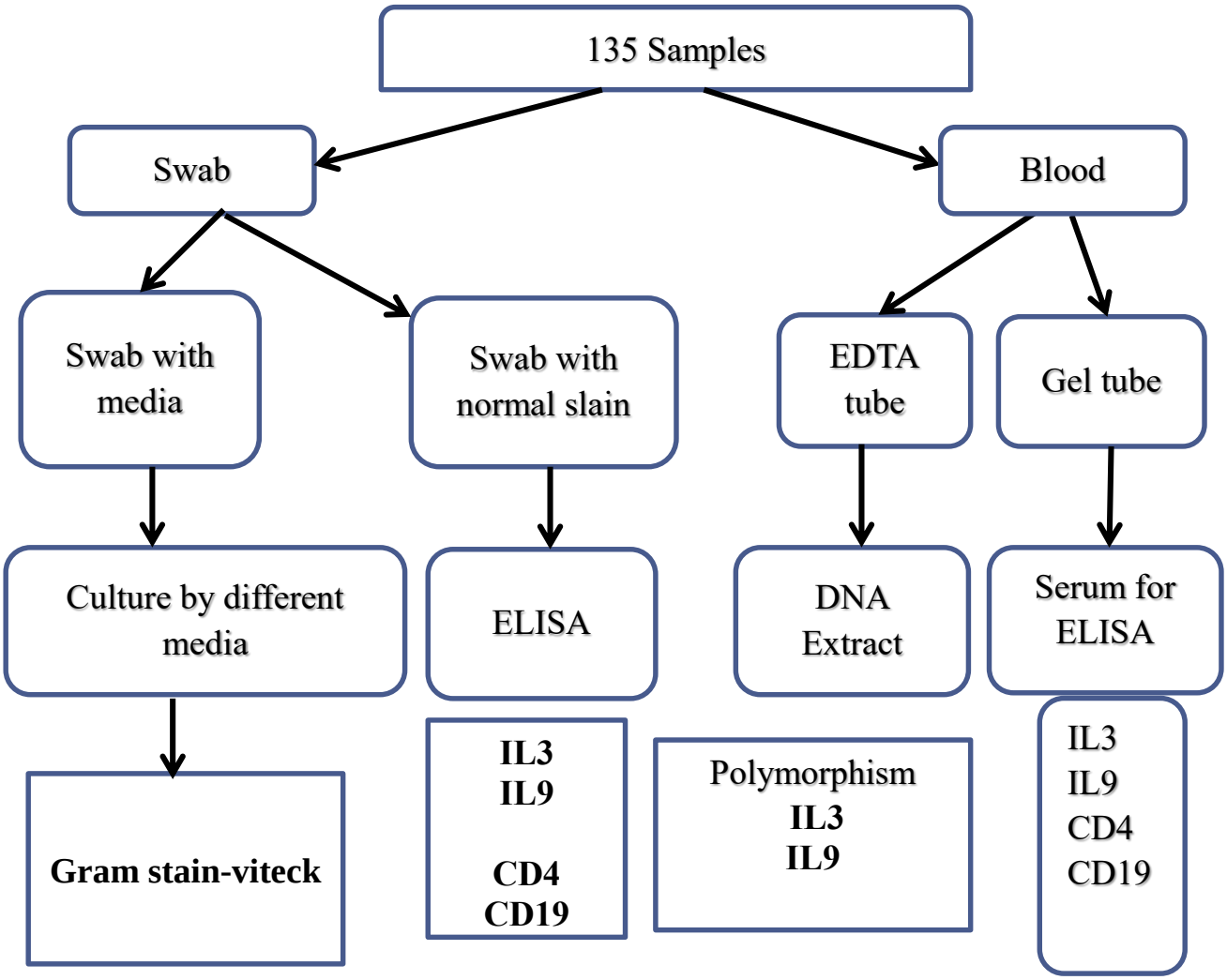


Figure (3-1) : Study Scheme Design

3-6 Laboratory diagnosis:

3-6-1 Isolation and identification of Gram positive and negative bacteria

A single colony was taken from each positive culture, negative culture and its identification depended on the morphology properties (colony size, shape, color and nature of pigments, translucency, edge, elevation and texture). Then, colonies were stained by Gram stain to observe a specific shape, type of reaction, aggregation and specific intracellular compounds (Winn *et al*., 2006).

3-6-2 Diagnostic tests

When the period of incubation was completed . colonies with differing characteristic were subjected to various test , and it were identified according to (Macfaddin ,2000 ; Forbes *et al*.,2007).

3-6-3 Vitek 2 system

The Vitek 2 System was used to confirm the result of the manual biochemical test, in recent times this system used to identify microorganisms(Winn *et al*. 2006). It was supplied with the required identification data base for all routine identification tests that provide an improved efficiency in microbial diagnosis which reduce the time and the need to do any additional tests , that will be safe for the user of system .

This system was performed according to the manufacturer's instructions (Biomerieux-France). This system consists of :

1- A personal computer.

2- Reader/incubator that consisting of multiple internal components including: card cassette, card filler mechanism, cassette loading processing mechanism, card sealer , bar code reader, cassette carousel and incubator.

3- The system also contains: transmittance optics , waste processing, instruments control electronics and firm ware .

This system was performed according to the manufacturer's instructions (Biomerieux-France):

1- Three ml of normal saline were placed in plane test tube and inoculated with a loop full of single colony of overnight culture.

2- The test tube was inserted into a dens check machine for standardization of colony to McFarland's standard solution (1.5×10 cell/ml).

3- The standardized inoculums were placed into the cassette.

4- Then a sample identification number was entered into the computer software via barcode. Thus the VITEK 2 card was connected to the sample ID number.

5- The cassette was placed in the filler module , when the cards were filled, transferred the cassette to the reader/incubator module.

1-Standardization

After primary isolation, handling is minimized in a simple inoculum preparation, standardization and dilution step,. The standardized inoculum is placed into the cassette and a sample identification number was entered into the computer software via barcode.

2-Traceability

The VITEK 2 card type is then read from the barcode placed on the card during manufacturing and the card is thus connected to the sample ID,. Manufacturer barcodes link the card to patient information in this one easy barcode reading step.

3-Load and Go

Place the cassette in the filler modu le. When the cards are filled, transfer the cassette to the reader/incubator module,. All subsequent steps are handled by the instrument.

3-7 Immunological assay ELISA technique

ELISA test was used for evaluation serum and swab CD4,CD19,IL3and IL9, concentrations for patient and healthy subjects as the following:

1. All reagents were prepared, standard solutions and samples as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.

2. Forty µl sample was added to sample wells and then add 10µl anti-antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells. Not blank control well. Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.

3. The sealer was removed and wash the plate 5 times with wash buffer. Soaking wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washed, aspirated all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

4. Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

5. Added 50µl stop solution to each well, the blue color will change into yellow immediately.

6. The optical density (OD value) was determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. The standard curve of all cytokines as in Figure (3-2, 3-3, 3-4, 3-5)

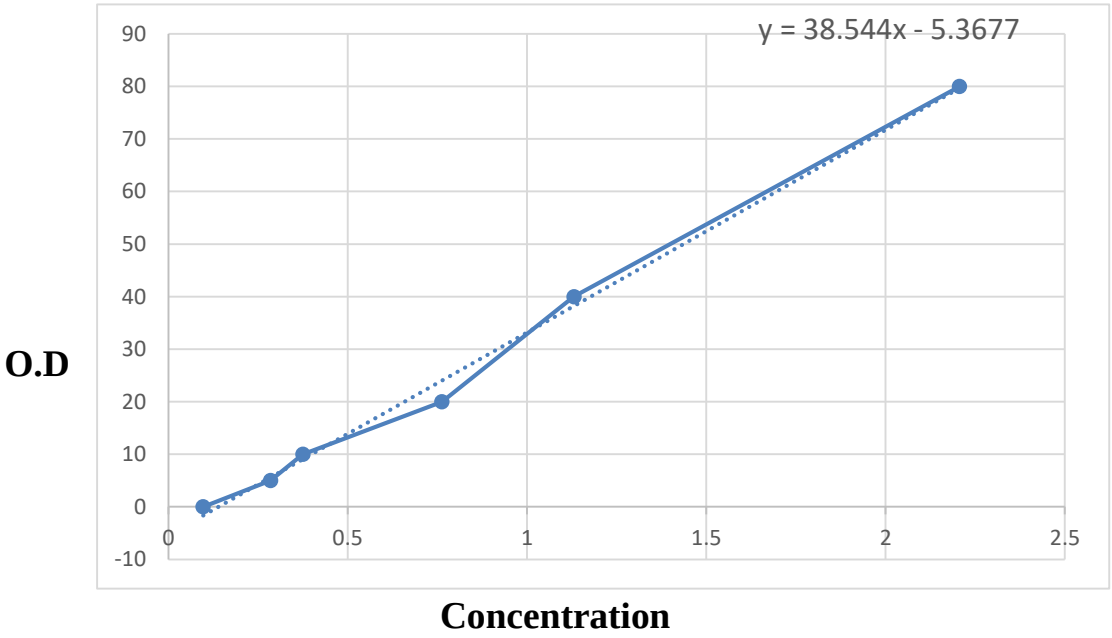
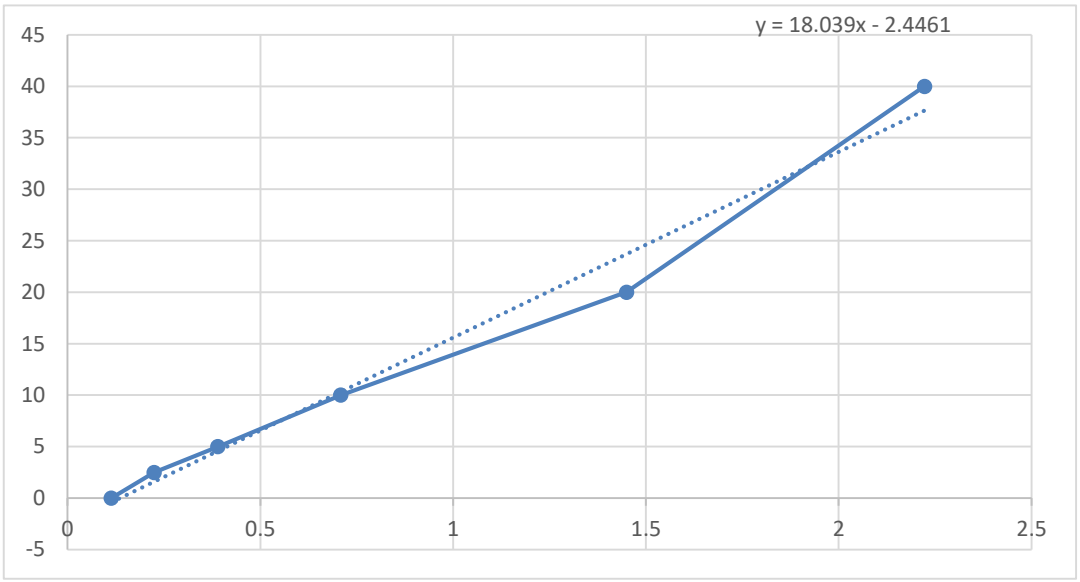


Figure (3-2): Standard curve of CD4

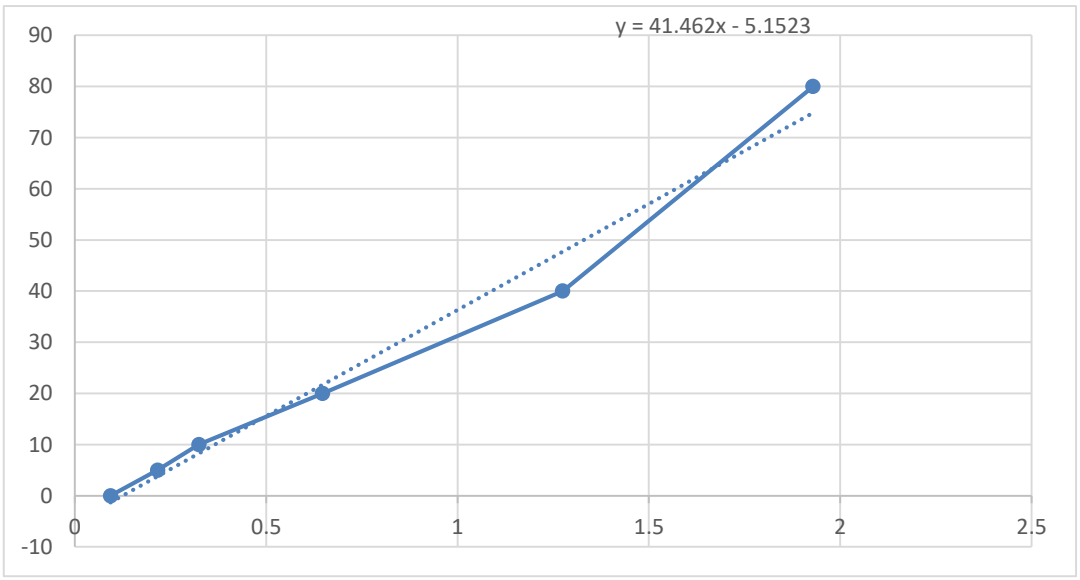
O.D



----------Concentration----------

Figure (3-3): Standard curve of CD19

O.D



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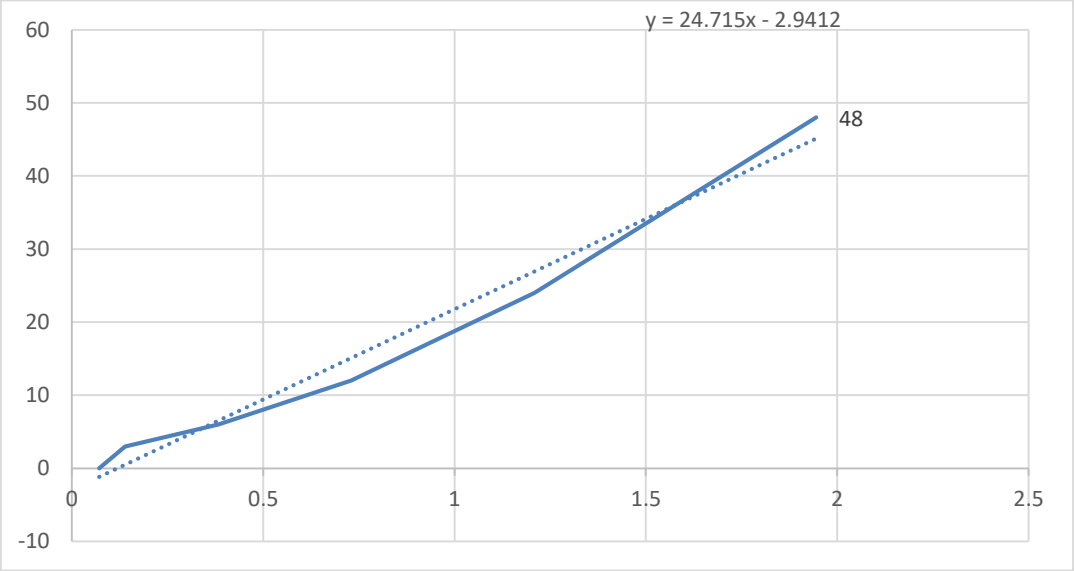
----------Concentration----------

Figure (3-4): Standard curve of IL3

O.D



----------Concentration----------

Figure (3-5): Standard curve of IL9

3-8 Genotyping assays:

3-8-1 DNA extraction

The DNA extraction was carried out at Department of Chemistry and Biochemistry, College of Sciences, in Kerbala city. DNA Genome was extracted from blood sample according to protocol AddPrep Genomic DNA Extraction Kit.

1.Principle.

Genomic DNA Extraction Kit is effective method for isolating genomic DNA from blood, tissue and plant. In case of extraction from blood, this kit is designed for the rapid preparation of genomic DNA from up to 200 µl of a blood sample like a whole blood, plasma, serum, buffy coat and body fluids, and is suitable to use with whole blood treated with either citrate or EDTA.

2.Procedure

1. A20 µl of Proteinase K solution (20 mg/ml) Were added to a 1.5 ml microcentrifuge tube.
2. Transferred 200 µl of sample to the 1.5 ml micro-centrifuge tube with proteinase K solution: If the sample volume is less than 200 µl, we were added the appropriate volume of PBS.
3. Then was added the 20 µl of RNase A solution (10 mg/ml).
4. Added 200 µl of Binding Solution to the sample tube, and mixed well by pulse-vortexing for 15 sec.
5. Incubated at 56℃ for 10 min: Longer incubation times have no effect on yield or quality of the purified DNA.
6. Added 200 µl of absolute ethanol and mixed well by pulse-vortexing for 15 sec: After this step, briefly spin down to get the drops clinging under the lid.
7. Carefully transferred the lysate into the upper reservoir of the spin column with 2.0ml collection tube without wetting the rim.
8. Centrifuge at 13,000 rpm for 1 min: Pouring off the flow-through and assembled the spin column with the 2.0 ml collection tube.
9. Added 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow through and assemble the spin column with the 2.0 ml collection tube.
10. Added 500 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow through and assemble the spin column with the 2.0 ml collection tube.
11. Dried the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.

12. Then transferred the spin column to the new 1.5 ml micro-centrifuge tube.
13. Added 100 ~ 200 µl of Elution Solution to the spin column with micro centrifuge tube, and let stand for at least 1 min.
14. Eluted the genomic DNA by centrifugation at 13,000 rpm for 1 min.

3-8-2 Determination the concentration and purity of the extracted DNA by nano droop

The extracted DNA was checked by using Nanodrop spectrophotometer ,which measured DNA concentration (ng/µL) (Adams, 2003**).** and check the DNA purity by reading the absorbance at (260/280nm) (Scientific, 2008)as following steps:

1. After opening up Nanodrop software, chosen the appropriate application (Nucleic Acid, DNA).

2. A dry wipe was taken to clean instrument pedestals several times. Then carefully pipette 2 µl of H_2O on to the surface of the lower measurement pedestals for blank system.

3. The sampling arm was lowered and clicked OK to initialized the Nanodrop, then cleaning off the pedestals and 1 µl of extracted DNA carefully pipette onto the surface of the lowered measurement pedestals,**.**

The concentration and A260/A280 ratio of DNA were documented from the instrument.

3-8-3 Primer dilution

Marcogene primers were commonly shipped in a lyophilized state. The units of a lyophilized primer are given as a mass, in Picomoles .To create a stock of primers, reconstituted was the primer in sterile 1X TE

(1mM Tris, mM EDTA, pH 8.0) .The company supplies the amount of TE or sterile nuclease-free d H_2O to be added to each primer to obtain master stock that would be used again to obtain working stock.

1-The tube was spin down before opening the cap.

2-The desired amount of water (300µl) was added according to the oligos manufacturer to obtain a master Stock. Vortex properly for re-suspend the primers evenly.

3-About 10µl was transferred of the master stock to a 0.2ml Eppendorf tube that contains 90µl of sterile, nuclease-free d H_2O (Working Stock).

4-The master stock and working stock was stored at -20 °C.

The working stock was thawed on ice and vortex before using in PCR and then stored at -20 °C. Once the primers are reconstituted and/or diluted, it is recommended that the primers should be distributed into single-use aliquots. Making single-use aliquots limits the freeze-thawing of primers and therefore will extend their life. It is recommended to store both primers at - 20 °C.

3-8-4 Amplification of DNA

The allele-specific PCR, which is also known as an ARMS- PCR (amplification refractory mutation system) or PASA (PCR amplification of specific alleles) or AS-PCR, was used to detect the SNPs (Darawi *et al*., 2013). Allele- specific PCR reaction protocol was used for SNPs detection *gene of IL3* and *IL9*. The Allele Specific –PCR reactions were performed in 25 µl volumes in PCR tubes under sterile conditions, all the volume of the reaction mixture was completed to 25 µl with using d H_2O and the master mix which contained optimum concentrations of reaction

requirements ($MgCl_2$ 1.5 mM, Taq polymerase 1 U, each dNTPs 200 µM) has been used,

3-8-5 Primers for PCR

A primer is a short single strand of DNA fragments consisting of (18-22) bases known as oligonucleotides that have a sequence that is complementary to the target DNA region. Without the use of primers, the amplification process cannot begin on a single DNA molecule. Thus, it should first be annealed to the single strands that result from the denaturation of the double stranded DNA (Chaitanya, 2013). Polymerase chain reaction was performed using a specific primer pairs designed for IL3 and IL9 gene. Based on NCBI database, all gene information and SNPs detail, were collected using Genius software designed.

Preparation of the Primers in the following Steps:

Materials: lypholized primers, sterile d H_2O

1. The tube was spin down before opening the cap.
2. Preparing master stock, pmoles/µl, the desired amount of sterile d H_2O was added according to the manufacturer to obtain a 100 pmoles/µl (Master Stock).
3. The tube was mixed properly to re-suspend the primers equally.
4. Preparing working stock, 10 p moles/µl, ten microliters of the master stock were transferred to a 0.5 ml eppendorf tube that contains 90µl of sterile d H_2O to obtain a 10 p moles/µl (Working Stock). 5. The master stock was stored at -20 C^o Table(3-12)(3-13).

Table (3-11): Allele Specific –PCR Program for Detection of IL-3

No.	Stage	Tem	Time	Cycle
1	Initial Denaturation	95	5min	35
2	Denaturation	95	30sec	35
3	Annealing	58	35sec	35
4	Extension	72	55sec	
5	Final Extension	72	5min	
6	Hold Phase	10	10	Hold

Table (3-12): Allele Specific –PCR Program for Detection of IL-9

No.	Stage	Tem	Time	Cycle
1	Initial Denaturation	95	5min	35
2	Denaturation	95	30sec	35
3	Annealing	60	35sec	35
4	Extension	72	55sec	
5	Final Extension	72	5min	
6	Hold Phase	10	10	Hold

3-9 Ethical approval:

The necessary ethical approval from ethical committee in Al-Hassan General Hospital and was obtained. Moreover, agreement from the family and patients for sampling.

3-10 Statically analyze

The data were statistically analyzed by SPSS software version24.T-test ANOVA, this study was case control study.

Chapter Four

Results and Discussion

4-1 Characteristics of study population

After being clinically diagnosed by a consultant physician between the months of March and September (2022), a total of 180samples (90blood and 90 swab) of tonsillitis patients(51) males and(39)females were collected from those suffering in the Ear Nose Tonsil (ENT) of AL-Hassan Al-mojtaba Educational Hospital in Karbala city. Patients ranged in age from 1 to 60 year. A total of 90samples collected as healthy control were group (45blood and 45 swab)

Two separate samples were taken, a swab and blood, from each patient and control, then transported directly by cool box to the microbiology laboratory for additional processing over the course of two to three hours.

4-2 Distribution of patients and control

4-2-1 According to the sex:

The study included 90 samples there were 51/90 (56.66%) male patients, 39/90(43.33%) female patients, 28/45 (62.22 %) male control and 17/45 (37.77 %) female control as it was aged ranged between 1-60 years as found in the Figure of (4-1) that show the distribution of tonsillitis patients and healthy control according to the sex .

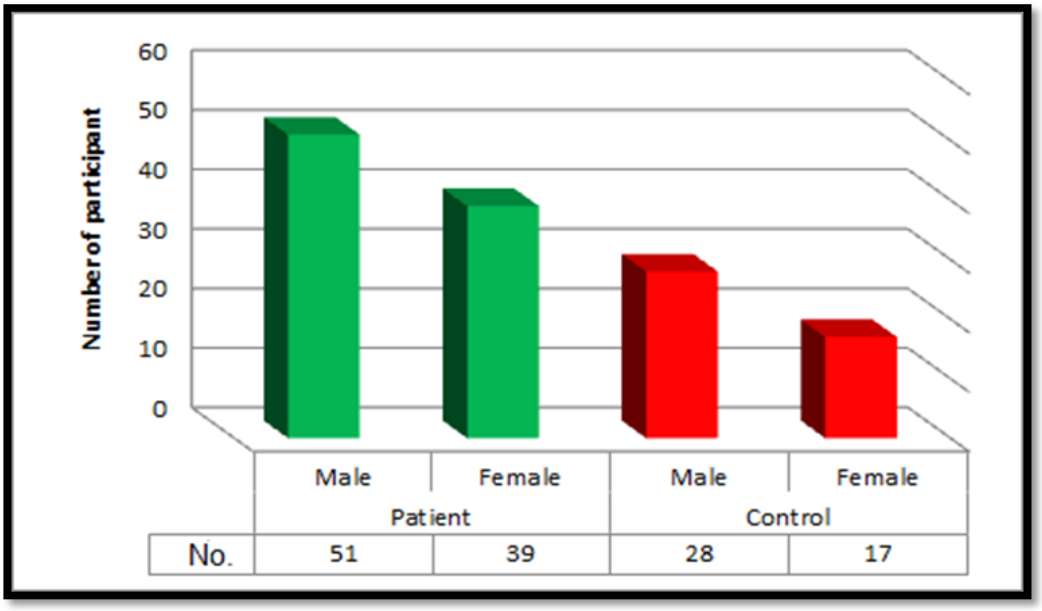


Figure (4-1) Distribution of tonsillitis patients and control according to the sex

This figure showed that the infection rate for male is higher than for female , this study agreed with Immunological and genetic studies with phenotypically were conducted on blood samples taken from patients and healthy participants The results of the current study showed that more tonsil infections were in males 51/90(56.6%) than in females 39/90(43.3%), In one study, the lifetime prevalence of recurrent tonsillitis is described as 11.7% (Kvestad *et al*., 2005).

This Figure demonstrated that males are more likely than females to contract the illness. This study contradicted one by(Farooqi *et al*. 2017) which found that tonsillitis was more common in female children than in male children.

The findings of the current study did not support those of (Jamal, 2015), who pointed that there were more females than males in a study on tonsillitis involving 200 patients. This might be the result of demographic differences (75 % patients and 25 % healthy control).

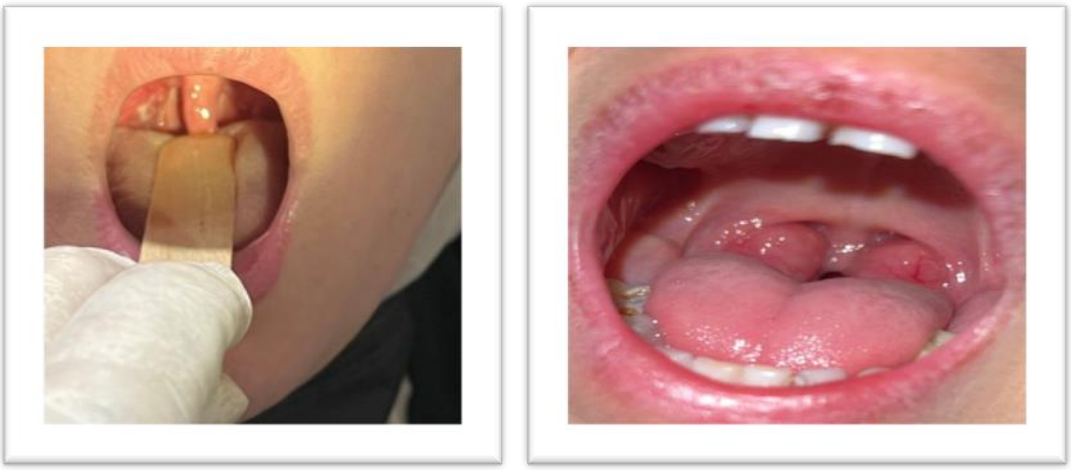
4-2-2 According to the type of infection:

According to physician diagnosis the percentage of acute tonsillitis was show a higher ratio of infection 48/90 (**53.3%)** followed by chronic tonsillitis 37/90 (**41.1%)** and recurrent tonsillitis 5/90 **(5.6%)** as shown in Table (4-1):

Table (4-1): Profile of patients according to the type of infection

Infection type	Distribution			%	p.value
	Female	Male	Total		
Acute infection	15	33	48	53.3%	
Chronic infection	12	25	37	41.1%	
Recurrent infection	2	3	5	5.5%	**0.75**
Total	29	61	90	100%	

The acute tonsillitis was appear with pus cell in picture (1)and a chronic infection (2) shown in Figure (4-2)



(1) **(2)**

Figure (4-2) Tonsillitis appearance in patients the (1) picture female suffer acute tonsillitis with pus cell appear and (2) picture male suffer chronic tonsillitis

4-2-3 Distribution of tonsillitis patients and control according to age group:

Ninety patients suffered from acute ,chronic tonsillitis and recurrent infection . The percentage in age (1-15) years was 17(18.80%) patients, while the percentage of a patient with age (16-30) years was 32(35.50%) patients. The percentage of (31-45) was 29(32.30%) patients, and (46-60) was 12(13.40%) patients,but The specimen of control was forty-five control. The percentage in age (1-15) years was 10(22.30%) persons. While the percentage of a patient with age (16-30) years was 11(24.50%) , (31-45) was12(26.60%) patients and (46-60) was 12(26.60%) the ratio of infection in the male more than in females in all population patients and control Table (4-2) .

Table (4-2) Distribution of tonsillitis patients and control according to age group

Age/Year	Patients NO.%	Control NO.%
(1 to 15)	17(18.9%)	10(22.2%)
(16-30)	32(35.6%)	11(24.6%)
(31-45)	29(32.2%)	12(26.7%)
(46-60)	12(13.3%)	12(26.7%)
Total	90 100%	45 100%
	X^2= 2.435 P= 0.487	

There tables showed that the infection occurs was high in aged group(16-30)years and the infection occur in male was high than female in all age group,

This study did not agree with a study obtained by (Farooqi *et al*., 2017) who showed tonsillitis was more common among female children as compared to male children .This might be due to different in

population. The results of study conducted by Farooqi appeared infection were highly in age group less than 10 years compare with other age group .These results of the present study were disagreement with (Al-Aawaadi, 2014) who recorded that the age group of <10 years the highest infection rate compared with other age but in the Table (4-2) most children (1-15) years was 18.8%, While percentage patient with age (16-30) years was35.5% The percentage of(31-45) was 32.3% and (46-60)was 13.4%, and also disagreed with (Dakhil&Hamim ,2016) who reported that age group of 1-10 years recorded the highest infection rates with`60 patients 65%, but the results of this study were agreement with (Agrawal *et al.*, 2014) that's recorded the age (11-29) years was35.5% group was the most affected with tonsillitis in this study .

4-3 Bacteriological study:

4-3-1 Diagnostic of bacteria that isolated from tonsillitis:

Based on culturing on different media, Gram stain, microscopic diagnosis, and confirmation by viteck, the isolation and diagnostic of microorganism from patients who had tonsillitis is shown in Figure (4-3).

Figure(4-3) Study culture media- Blood ,MacConkey and Chocolate agar

4-3-2 Percentage of bacteria that causes of tonsillitis according Gram stain:

Percentage of bacteria causes tonsillitis from the 90clinical specimens. 61**(67.7%)** specimen isolated were belong to Gram positive bacteria and 29(32.2%) specimen isolated were belong to Gram negative bacteria as shown in the Figure (4-4):

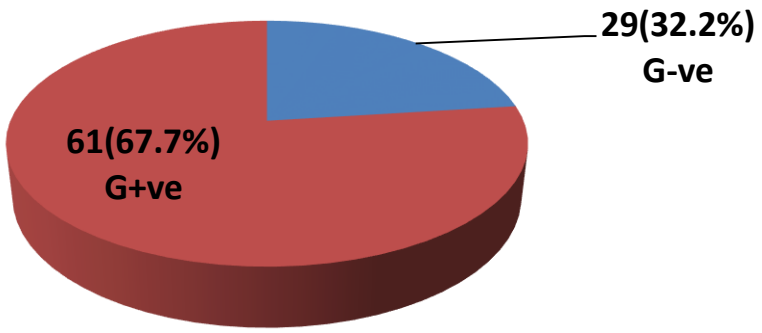


Figure (4-4) Percentage of bacteria that causes of tonsillitis according Gram stain

Tonsillitis involved growths were identified using enriched and differential media (Blood ,MacConkey and Chocolate agar)Figure(4-5) showed Gram stain technique, as well as to identified type of bacteria after that select the disc of vitek.

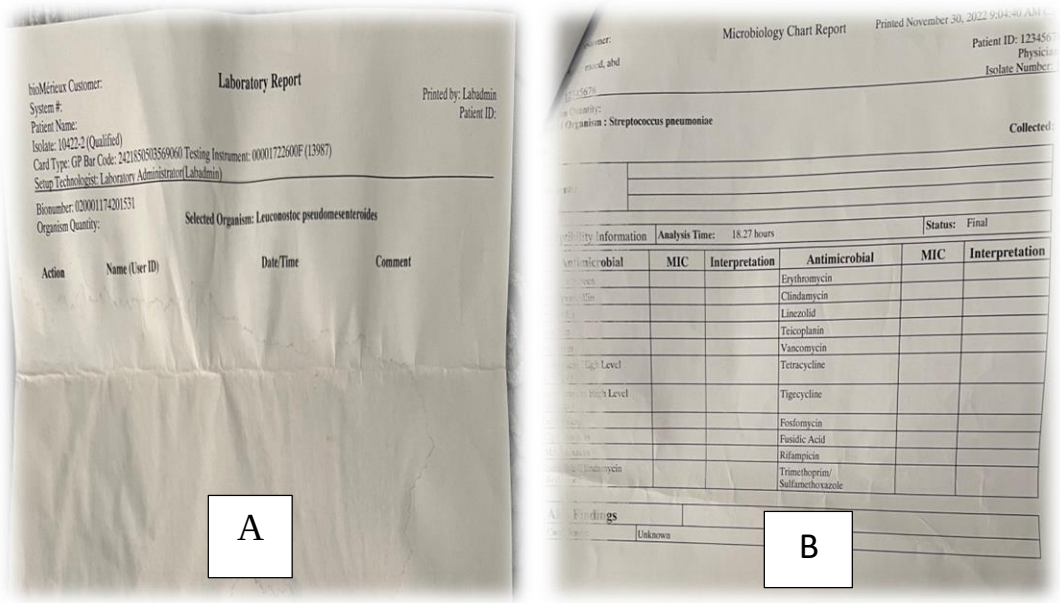


Figure (4-5) Some vitek results in the (A)picture result of vitek for patient suffer *Leuconostocpseudomesenteroides* bacteria (B) picture result of vitek for patient suffer *Streptococcus pneumonia* bacteria

As show in Table (4-3) this study included isolation of different species of Gram positive bacteria isolated from tonsillitis patient. The high percentage of *Streptococcus pyogenes* 24/90(26.6%) followed by *Staphylococcus aureus*9/90(10%),*Staphylococcuse pidermidis*8/90(8.8%) *Staphylococcus haemolytic* 7/90 (7.7 %), *Streptococcus pneumonia*6/90 (6.6 %), *Staphylococcus lantus* 4/90 (4.4%)

And 1/90(1.1%) of each, *Buttiaurella agrestic* , *Staphylococcus sciuri* and *Leuco*. *pseudomesent* While Gram negative bacteria, the high percentage 11/90 (12.2%) in *Klebsiella pneumonia* followed by *Escherichia coli* 8/90(8.8%),*Pseudomonas aeruginosa*4/90 (4.4%) *Pseudomonas fluorescence*3/90(3.3%)and1/90(1.1%)ofeach*Enterobacter acrogens*, *Legionella pneumophila*, and *Acinetobacter urisingii*.

Table (4-3) The percentage of bacteria isolated from tonsillitis swab

Type of bacteria	Bacteria	Number specimen (No)	Percentage %
Gram positive			
	Buttiaurella agrestic	1	1.1%
	Leuconostoc.pseudomesenteroides	1	1.1%
	Staphylococcus haemolytic	7	7.7 %
	Staphylococcus aureus	9	10%
	Staphylococcus epidermidis	8	8.8%
	Staphylococcus lantus	4	4.4%
	Staphylococcus sciuri	1	1.1%
	Streptococcus pneumonia	6	6.6%
	Streptococcus pyogenes	**24**	**26.6%**
Gram negative			
	Acinetobacter urisingii	1	1.1%
	Enterobacter acrogens	1	1.1%
	Escherichia coli	8	8.8%
	Klebsiella pneumonia	**11**	**12.2%**
	Legionella pneumophila	1	1.1%
	Pseudomonas aeruginosa	4	4.4%
	Pseudomonas fluorescence	3	3.3%
	Total	**90**	100%

Table (4-3) showed the most common infection in acute ,chronic and recurrent tonsillitis was *Streptococcus pyogenes* as positive bacterium with proportion reach to 26.6%, on the other hand, the most common infection of bacteria Gram negative was *Klebsiella pneumonia* with proportion reached to 12.2% , the inflammation usually extends to the adenoid and the lingual tonsils; therefore, the term pharyngitis may

also be used. Most cases of bacterial tonsillitis are caused by group A beta-hemolytic *Streptococcus pyogenes* (GABHS).

Other time, the most common organisms associated with tonsillitis are also the normal microflora of the mouth (*Porphyrmonas spp*., *E. coli*, *Staphylococcus aureus*) (Esposito *et al*., 2014).

In the present study found *Streptococcus* most common bacteria this agree with other local study in Kirkuk city in there study found *Streptococcus*36.73% while *Staphylococcus* 30.6%(Ali and Mahdi,2016). and also similar with other study in Mosul city in there study found that *Streptococcus* 81.53% while *Staphylococcus*44.6%(Alloe *et al.*,2009).

This study agree with other broad study in India in these study found that beta hemolytic *Streptococcus* (51.4%) (Vijayashree *et al*., 2014) and Also similar with study in India in these study found *S. pyogenes* was the commonest isolate, followed by *S. aureus* (Sridevi *et al*.,2016), Similar with study in Pakistan in there study and they were concluded that tonsillitis have variable etiologies but most common are bacterial & viral infections especially *S. pyogenes* (Agreen *et al* ;1998)

The present study was not similar with study of (Mahajan &Ingale, 2017). in their study found that *Staphylococcus* over *Streptococcus.* Also the result show that *S. aureus* 18% and *K. pneumonia*1%this like with other study in UAE in there study isolated of *S. aureus*.9.5% and 1.9% of *K. pneumonia* from tonsil (Cherian *et al.*, 2012). However study in Erbil, Iraq reported that the most frequent isolated microorganisms from tonsillitis/pharyngitis patients were *S. aureus* 54 (30.5%), *S. pyogenes* 16 (9%), *S. parasanginis* 12 (6.8%), *P. aeruginosa* 11 (6.2%) and *Proteus mirabilis* 10(5.6%) (Bakir and Ali,2015).

E. coli, p. aeruginosa, .Staphylococcus and *Streptococcus* in this study similar with other study in Benin City in there study found that 19 (48.72%) were ß haemolytic *Streptococcus*(BHS). others were *S. aureus* 5(12.83%), 7(17.95%) were *P. mirabilis* and 3(7.69%) each of *P. aeruginosa* and *P. mirabilis*(Wilson *et al* .,2008).

E. coli was isolated from 6 cases and *K. pneumonia* were isolated from 2 case the result was closely to the several study that isolated Enterobacteriaceae from throat swabs (Kurien *et al.*,2000).But did not similar with a study findings by (Cherian *et al*.,2012) that do not isolated of *E.coli* from tonsil swabs when isolated of *K. pneumonia* from tonsil swab similar with these study .

Pseudomonas show in 4 isolated (4.3%) and this percentage similar to another study in Erbil (Bakir and Ali,2015) and agree with study (Agrawal *et al.;*2014) and other Gram negative species such as *Acinetobacter ursingii, Acinetobacter iwoffii , Acinetobacter baumonia ,Pantoea spp, Acinetobacter baumanni ,Klebsiella oxytoca Buffiauxella agrestic, Oligella ureolytica* there is available reference to compare this result with it

The unusual presence of Gram negative G-ve coliform organisms (*Klebsiella*, and *Pseudomonas*) in this study is noted. Since they are not traditional upper respiratory pathogens, it is possible that they could have been transferred from the anal region to the throat following poor personal hygiene . May be causes other infection of patient such as septicemia and other disease .

Other study show *S. aureus* 54 (30.5%), *S. pyogenes* 16 (9%), *S. parasanginis* 12 (6.8%), *P. aeruginosa* 11 (6.2%), and *Proteus mirabilis* 10 (5.6%) were the most commonly isolated microorganisms from

tonsillitis/pharyngitis patients, according to a study done in Erbil, Iraq (Bakir and Ali,2015).

The results were at odds with a number of investigations that found 8 and 10 cases of enterobacteriaceae isolation from throat swabs, respectively (Kurien *et al*., 2000; Al – Galil *et al*.,2014).

4-4 Characteristic the bacteria isolation

Based on culturing media, Gram stain, microscopic diagnosis, and confirmation by viteck, the isolation and diagnostic of some microorganism from patients who had tonsillitis is shown in Table (4-4).

Table (4-4) : Characteristics of some bacterial isolate on media

Bacterial type	Colony shape on	
Gram positive	**Blood agar**	**Maconkey**
Escherichia coli	Smooth (fresh isolation) ; rough repeated subculture; mucoid capsulated; strains. Greyish white color β Hemolysis (in some strains)	Smooth (fresh isolation) ; Rough (repeated subculture) ; mucoid (capsulate d strains), pink color

Leuconostoc.pseudomesenteroides	Gray to whitish colonies surrounded by a narrow zone of β- hemolysis	No growth
Staphylococcus epidermidis	a white pigment, non-hemolytic.	No growth
Staphylococcus lantus	Not blood hemolysis	No growth
Staphylococcus aureus	Colonies are circular ,convex ,smooth ,shining,opaque, emulsified easily, beta-hemolysis.	No growth
Strepticoccus pyogenes	A clear &complete hemolysis	No growth
Streptococcus pneumonia	Mucoid appearing as a central depression, alpha hemolysis	No growth
Klebsellia pneumonia	Colonies are non-hemolytic and mucous colony	pink colored due to the lactose fermentatio n

Pseudomonas aeruginosa	B-hemolytic, sometime producing asweet or grapelike odor	Round, flat andcolorless colonies indicating thatthe organism isa lactose non fermentation
Pseudomonas fluorescence	No distinctive appearance	Unable to degrade lactose,nocolor change according to the pH indicator natural red, colorless to pink colonies

4-5 Immunological parameters study:

4-5-1-: Interleukin 9 concentration in serum and swab

In this study showed that there was a large difference ($P \leq 0.05$) in the serum levels of IL9 between patients and healthy controls. The IL9 serum levels were increased in tonsillitis patient mean having a level of (13.07±4.92) pg/ml while the healthy controls having a level of (10.74±4.66) pg/ml at p value0.163 . Also in the swab sample , IL-9 concentration was higher in the patient (12.91±3.35) compare with the healthy controls (10.18±3.94) pg/ml at p value0.026 Table (4-5). The

concentration of IL-9 high mean increased concentration between serum and swab in all patients and control.

Table (4-5) Concentration of IL-9 pg/ml between tonsillitis patients and control in serum and swabs

IL-9 pg/m	NO.	Mean ±SD	P value
Patient Serum	90	13.07±4.92	0.163
Control Serum	45	10.74±4.66	
Patient Swab	90	12.91±3.35	0.026
Control Swab	45	10.18±3.94	

(Pasvenskaite *et al*.,2021) support this finding show 20 control subjects and 20 laryngeal squamous cell carcinoma (LSCC) patients had their serum IL-9 levels checked. Then, all 40 subjects' results were collected. However, with a mean of 8.99 12.03 pg/mL and a measuring range of 3.1-200 pg/mL and sensitivity of 0.5 pg/mL.

(Dantas *et al* .,2015) were find the serum levels of IL-9 in patients with Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) were significantly higher than in healthy individuals. There was no difference between the levels in SLE patients and RA patients. There were no statistically significant associations or correlations between the levels of IL-9 and SLEDAI, the number of Albumin Creatinine Ratio (ACR) criteria, organ damage, clinical manifestations, complement consumption, anti-nuclear antibodies (ANA), or anti-DNA positivity, with the exception of disease duration, which displayed a statistically significant negative correlation with IL-9 levels.

4-5-2 Interleukin -3 concentration:

IL-3 cytokine were estimated by using Enzyme Linked Immunosorbent assay (ELISA) were used for quantification of human IL-3 the result of IL-3 its shown serum levels were significantly decreased in tonsillitis having a level of (9.119±2.66) pg/ml while the healthy controls having a levels of (11.31±2.98) pg/ml at p value0.049. Also in the swab, IL-3 concentration in the patient (5.69±1.107) compare with the healthy controls (8.76±2.18) pg/ml at p value0.001 as found in Table(4-6).

Table (4-6) Concentration of IL-3 between tonsillitis patients and control in serum and swab samples

IL-3 pg/m	NO.	Mean ±SD	P value
Patient Serum	90	9.119±2.66	0.049
Control Serum	45	11.31±2.98	
Patient Swab	90	5.69±1.107	0.001
Control Swab	45	8.76±2.18	

(Kunnath-Velayudhan *et al*., 2021) investigated were infected intravenously whether the increased bacterial burden seen in IL-3 deficient mice compared to wild type (WT) mice was related to increased lung pathology or decreased survival after *Mycobacterium tuberculosis* infection. Their findings supported those of the current study. Because IL-3-secreting T helper cells were only activated when the infection occurred at epithelial barriers, it is likely that no difference in survival was seen when mice were infected intravenously. Overall, these investigations showed that IL-3 provided limited but detectable protection against infection with *M. tuberculosis* in common mouse models of this infection.

(Fu *et al* .,2016) significant variations in IL-3 levels were found between FEDN patients, chronic patients, and healthy control participants

after using a natural logarithmic transformation. The difference between these three groups continued to be significant even after potential confounding covariate characteristics including age, gender, smoking, and education were taken into consideration. In comparison to controls, IL-3 levels were considerably lower in FEDN patients than in control. participants.

4-5-3 Cluster of differentiation(CD4) concentration

Cluster of differentiation CD-4 cytokine was estimated by using (ELISA) test was used for quantification of human CD4 the result of this test was calculated by using standard curve fit equation. The mean CD4 concentration in the serum of the patient was (22.11±3.66) pg/ml while the control was (17.39±1.38) pg/ml with high mean at p value0.038 . Also in the swab patient (21.97±1.53),and the swab control (15.76±1.97) was higher in the patient compared with the control at p value0.029 . The high mean and increased concentration between serum and swab in all patients and control CD4 cytokine and giving increased in p.value >0.05 as show in Table (4-7).

Table (4-7) Concentration of CD-4between tonsillitis patients and control in serum and swab

CD4 pg/m	NO.	Mean ±SD	P value
Patient Serum	90	22.11±3.66	0.038
Control Serum	45	17.39±1.38	
Patient Swab	90	21.97±1.53	0.029
Control Swab	45	15.76±1.97	

(Tseng, *et al.*,2013) confirms the findings of the present research They examined patients with Systemic lupus erythematosus (SLE), Rheumatoid arthritis (RA), and Ankylosing spondylitis (AS), which are

well-known autoimmune diseases with typical chronic inflammation and leukocyte activation, to determine whether the serum sCD4 level is relevant to disease progression. Patients with SLE and RA exhibited much greater quantities of sCD4 than the control participants, which is why gout patients who represent acute inflammation are also included for comparison. Although not statistically significant compared to controls, serum sCD4 levels were also significantly higher in the AS patient group. RA and SLE patients had higher serum sCD4 levels, suggesting a connection to disease activity.

(French, *et al.*,2011) are supported by the possibility that indicators of defective effector memory CD4 T cells and B cells in HIV-infected patients with elevated CD4 T cell numbers on antiretroviral therapy can predict vulnerability to opportunistic infections. Even when their CD4 T-cell counts have increased, some HIV patients who had severe immunodeficiency before starting ART continue to be vulnerable to opportunistic infections (OIs). A risk of 1.75 occurrences per 100 person-years across a median follow-up period of 184 weeks for patients who had previously experienced a nadir CD4 T-cell count below 50 cells/mL (range 8–216 weeks). More than 40% of events took place at a higher CD4 T-cell level than was anticipated.

4-5-4 Cluster of differentiation(CD19) concentration

ELISA quantification of human CD19 was calculated by using a standard curve fit equation . The mean concentration of CD19 in patient serum was(49.34±3.57) pg/ml while the control was(22.81±6.59) ng/ml with a high significance at p.value 0.022 . Also in the swab, CD19 concentration was higher in the patient (47.33±2.91) compare with the control (25.9±3.83) ng/ml concentration at p.value 0.019 . The highly

significant and increased concentration between serum and swab in all patients and control and giving increased in p.value >0.05 as show in Table (4-8).

Table (4-8) Concentration of CD-19 between tonsillitis patients and control in serum and swab samples

CD19 ng/ml	NO.	Mean ±SD	P value
Patient Serum	90	49.34±3.57	0.022
Control Serum	45	22.81±6.59	
Patient Swab	90	47.33±2.91	0.019
Control Swab	45	25.9±3.83	

(Alhasnawi, & Aljanaby .,2022) in line with the findings of this study, it was shown that patients with stomach cancer and *Helicobacter pylori* had significantly higher serum concentrations of CD19 and Galactin-3 (P-value 0.0001) compared to controls. Moreover, compared to *H. pylori*-free patients, there was a substantial rise in CD19 (P-value = 0.0035) and Galectin-3 (P-value = 0.0022) in stomach cancer patients..

(Wang *et al*.,2017) supported the current study, which found that patients with Systemic Lupus Erythematosus (SLE) had considerably greater percentages of CD19+CD38 high CD24 high B cells in their peripheral blood mononuclear cells and B cells than did healthy people. According to this study, patients with SLE had Bcells that were significantly enriched in CD19+CD24 high CD38 compared to healthy people. A similar amount of CD19+CD38 high CD24 high B cells were found in both SLE patients and healthy people, according to this study, but the CD19+CD38 high CD24 high B cells in SLE patients were functionally compromised.

4-6 Correlation between serum(systematic)and swab(locally) concentrations CD4, CD19.IL3 and IL9 in patient with tonsillitis infection and healthy control

4-6-1 Correlation between parameters with patients

The concentration levels of some patient cytokines were estimated in the samples studied using the enzyme-linked immune sorbent assay technique automated ELISA, and the correlation (r) in the cytokines between serum and swabs were determined with probability significant show in Table (4-9).

Table (4-9) Correlation between serum and swab concentration by ELISA in patient

Patient	IL-3 swab	IL-3 serum	IL-9 swab	IL-9 serum	CD4 swab	CD4 serum	CD19 swab	CD19 serum
IL-3 swab	0	0.03	0.643	0.585	0.647	0.642	0.876	0.486
IL-3 serum	**-0.378**	0	0.692	0.126	0.631	0.003	0.81	0.027
IL-9 swab	-0.084	0.072	0	0.702	0.018	0.7	0.003	0.741
IL-9 serum	0.1	0.276	-0.07	0	0.013	0.093	0.769	0.014
CD4 swab	-0.083	-0.087	0.408**	-0.434	0	0.912	0.575	0.498
CD4 serum	-0.084	0.497	0.07*	0.302**	-0.02	0	0.788	0.005
CD19 swab	0.028	-0.044	0.507**	0.054	-0.101	0.049	0	0.077
CD19 serum	-0.126	0.386*	0.06	0.431*	0.122	0.48*	-0.313	0

Table (4-8) show*Black color correlation coefficient (r) ; blue colors significant in the p values , yellow color significant P< 0.05 in patient the study recorded significant (P= 0.03) weak positive correlation (r=0.386).

In this study appearing positive correlation between (CD4swab and IL9swab),(CD4serum and IL9swab and IL9serum),(CD19swab and IL9swab),(CD19serum and IL3serumand IL9serum and CD4serum).

IL-9 is a pleiotropic cytokine produced primarily by Th2 helper T cells. It supports the growth of activated T-cell subsets and synergizes with IL-3 cytokines .In the mouse model of eosinophilia tissue inflammation, IL-9 acts by promoting an influx of eosinophil's and enhancing their differentiation, maturation, and survival (Pajulas *et al*., 2022).

The researchers found mid positive correlation (0.507) between 1L-9 cytokines with CD19 in patient saliva with significant association (P= 0.03), B-lymphocyte antigen CD19, also known as CD19 molecule (Cluster of Differentiation 19), B-Lymphocyte Surface Antigen B4, T-Cell Surface Antigen Leu-12 and CVID3 is a trans membrane protein that in humans is encoded by the gene CD19. In humans, CD19 is expressed in all B lineage cells (Wang *et al*., 2012).

CD19 plays two major roles in human B cells: on the one hand, it acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane; on the other, it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways, due to its presence on all B cells, it is a biomarker for B lymphocyte development (Maggini et *al*., 2018).

The researchers found mid positive correlation (0.48) between CD4 cytokines with CD19 in patient serum with significant association (P= 0.05), some of study found the transcription of CD4 mRNA in CD19-positive lymphocytes was suppressed by infection with the B95-8 strain of EBV and lost in B95-8-transformed (Zhang & Henderson, 2012).

4-6-2 Correlation between parameters with healthy control

There is no correlation between all parameter IL3,IL9,CD4and CD19of healthy control group Table (4-10).

Table (4-10) Correlation between serum and swab concentration by ELISA in control

Control	IL-3 swab	IL-3 serum	IL-9 swab	IL-9 serum	CD4 swab	CD4 serum	CD19 swab	CD19 serum
IL-3 swab	0	0.441	0.509	0.583	0.999	0.701	0.676	0.161
IL-3 serum	0.246	0	0.197	0.765	0.151	0.197	0.611	0.311
IL-9 swab	0.212	0.401	0	0.258	0.232	0.982	0.451	0.062
IL-9 serum	-0.177	-0.097	-0.354	0	0.814	0.152	0.31	0.695
CD4 swab	0.123	0.442	0.374	-0.076	0	0.256	0.722	0.699
CD4 serum	0.09	0.401	0.007	0.44	-0.356	0	0.979	0.195
CD19 swab	0.135	0.164	0.241	-0.32	0.115	-0.08	0	0.684
CD19 serum	0.432	0.32	0.554	0.127	0.125	0.402	0.131	0

4-7 Genotyping study

The genomic DNA was extracted from the blood samples (90) patient and (45) control as a first step to amplify the target region of IL3 and IL9.

4-7-1 *IL-3 gene* Polymorphism "rs40401" detection by polymerase chain reaction:

The study was used allelic specific PCR to identify types of polymorphism .The subjects enrolled in present study were classified into three genotypes; one homozygous for the C allele (CC) wild type, heterozygous (CT) and the last was homozygous for the allele T (TT).

The results of the current study for genotype distribution of the (rs40401) SNP exhibited a two band 337 , 337 bp for CT, and one band for each CC 337 bp, and TT 337 bp genotype for all participants Figure (4-6)for patients and Figure (4-7)for healthy control .

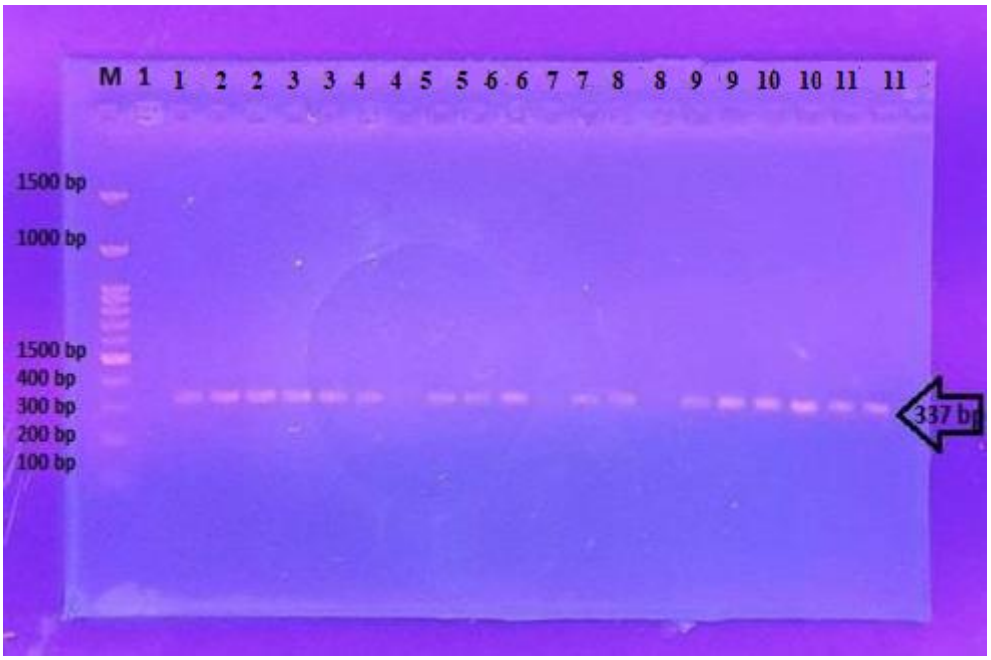


Figure (4-6) Agarose gel electrophoresis image that showed the Allele specific-PCR product analysis of *IL-3 (rs40401*) Gene polymorphism for patients ;

M represented DNA marker, , 11 well represented as Wild allele ; 2, 3, 5, 9 and 10 well represented as Heterozygous alleles 1 ,4 and 6 wells represented as homozygous mutant alleles. a ladder pattern (337bp)Electrophoresis conditions, 1.5% agarose, 70V, 20mA for 1h, stained with red safe dye**.**

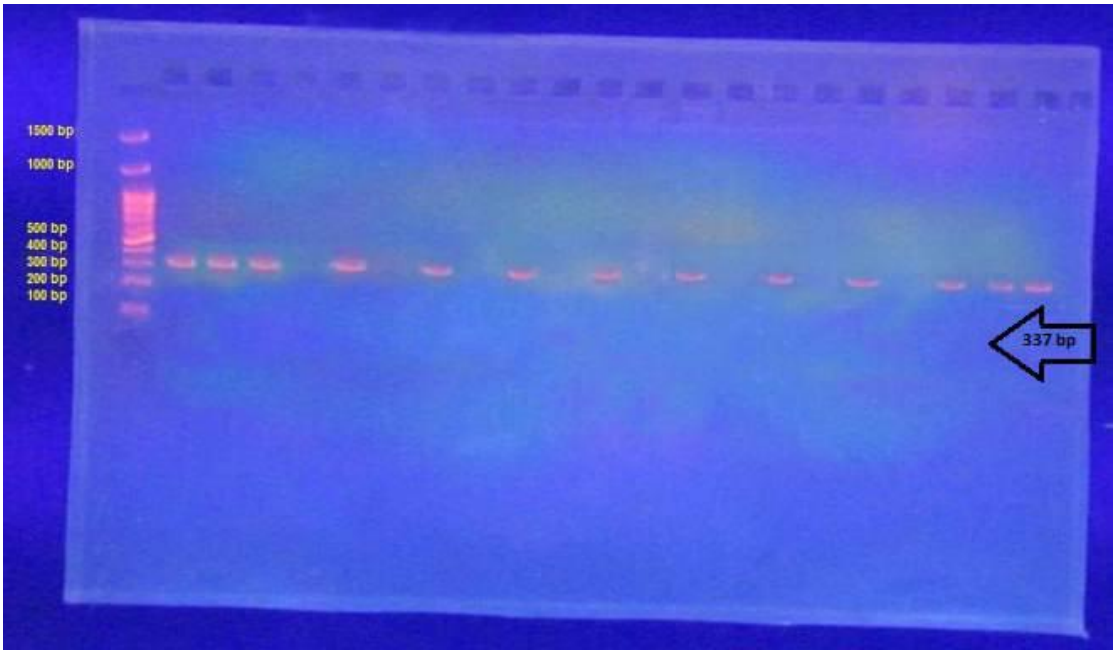


Figure (4-7) Agarose gel electrophoresis image that showed the Allele specific-PCR product analysis of *IL-3 (rs40401)* Gene polymorphism for control ;

M represented DNA marker, 2 , 3,4,5,6 ,7,8,9 and11 well represented as Wild allele ; 1 and 10 well represented as Heterozygous alleles, a ladder pattern (337bp)Electrophoresis conditions, 1.5% agarose, 70V, 20mA for 1h, stained with red safe dye.

4-7-2 Allele and genotype frequencies for rs40401 polymorphisms in the study population:

The IL3 SNP rs40401 variant were investigated in this study. All of this polymorphism, was observed in this Iraqi population Table(4-11) and were consistent with Hardy–Weinberg equilibrium, the variant alleles of *genes IL3 SNP rs40401* (T allele) occurred with a lower frequency than the wild-type allele (C allele), The frequencies of the IL3 SNP rs40401 alleles were C = 0.65, T = 0.35; A relatively large frequency of the population was heterozygous (No.=45) more than wild and mutation alleles.

Table (4-11) Genotype and allelic frequency of *IL-3 gene* polymorphism among study patients

Genotype		No.of participants	Frequency HWE
Wild	CC	36	0.4
Hetero	CT	45	0.5
Homo	TT	9	0.1
Total		90	1
Alleles			**Frequency**
C allele		117	0.65
T allele		63	0.35
p.value		180	1
Statistical analysis		X^2= 0.288 Consistent with HWE	

The Hardy–Weinberg principle can be used to infer the evolution of species when it is violated. Non-random mating, mutations, selection, small population size, random genetic drift, and gene flow are some examples of disturbing influence (Wise, 2018).

The results of the study showed in Table (4-12) the presence of three genotypes related to IL3 SNP rs40401 variant in study patients and their relationship with the concentrations of the four cytokines, which include IL-3, IL-9, CD4 and CD19 in their blood; the study found significantly increased (P< 0.05) just in cytokines IL-3 and IL-9 in homozygous wild patient, they are recorded as 15.29±1.43 and 17.84±1.57 pg/ml respectively. Otherwise, the study don't found any association significant between three genotypes with CD4 and CD8 cytokines.

Table (4-12): Concentration of cytokines among patient with IL-3 polymorphisms

Genotype Frequency rs40401 patients		IL-3	IL-9	CD4	CD19
Wild	CC N= 36	15.29±1.43	17.84±1.57	19.45±1.18	5.31±0.956
Hetero	CT N= 45	14.4±1.62	13.54±1.78	19.45±	7.49±
Homo	TT N= 9	12.17±2.29	18.07±1.96	21.42±1.27	6.46±1.18
Total	90	0.035	0.047	0.194	0.263

***Yellow color mean significant association p< 0.05**

Because of the nature of interleukin from the presence of the wild gene C allele, it gives the free nature of interleukin because the mutation T allele is of an unsatisfactory type and give new protein (missense change c.79C>T(p.Pro27Ser). Where interleukin is in its natural form as long as it contains protein organic acid classed as a proteinogenic amino acid (Proline) in the position of 27 sites (Werren *et al*., 2021).

IL-3 is able to bind to a receptor that is made up of two subunits: the specialized IL-3R subunit, and the ubiquitous c subunit. IL-3 is a multi-lineage colony-stimulating factor (CSF) because it works in conjunction with other cytokines to stimulate the development of immature progenitor cells of all lineages. It helps cells like macrophages, mast cells, and megakaryocytes live longer by preventing their death and promoting their survival. The majority of IL-3 is generated by lymphoid cells, however it can also originate from mast cells and eosinophils (Tsai *et al*., 2022).

(Baqer *et al*., 2022) explained that SNP of IL-3 “rs40401”has not been shown to be a risk factor in women with toxoplasmosis who have experienced recurrent abortions, despite the fact that the levels of IL-3 in

his patients' serum varied according to genotype and showed substantial variations from those of his controls.

This investigation was consistent with the findings of (Miyake *et al*., 2013), who discovered a strong positive correlation between a variant of the IL3 SNP called rs40401 and the chance of developing rhinoconjunctivitis in Japanese women.

Some studies showed that there is a significant correlation between IL-3 and IL-9 by studying them with some incurable diseases, There is substantial evidence to suggest that the immune response cytokine genes [interleukin (IL) -3, IL-9, is connected to autoimmune thyroid problems in the Chinese and Japanese people who have Graves' disease. autoimmune disease (Zhu *et al*., 2010).

The study did not notice any clear significant differences in each of the four cytokines IL-3, IL-9 CD4 and CD19 in relation to healthy subjects who they have genotype frequency "rs40401" among there chromosomes as in table (4-13).

Table (4-13): Concentration of cytokines among control with IL-3 polymorphisms

Genotype Frequency rs40401 control		IL-3	IL-9	CD4	CD19
Wild	CC N= 39	17.56±3.18	14.29±2.08	14.13±3.57	3.19±0.75
Hetero	CT N=4	16.57±2.09	14.18±2.62	15.32± 3.67	4.29±0.65
Homo	TT N= 2	17.06±3.18	15.33±2.11	16.43±3.81	5.19±0.38
p.value	45	0.834	0.645	0.223	0.448

Although the presence of the wild gene outweighs the other alleles heterozygous and homozygous mutation, we have obtained 2 healthy people who have the mutation of IL-3 type "rs40401", the reason for the presence of these mutations may be attributed to the fact that we did not properly confirm about healthy people, it may be attributed to the presence of clinical diseases that are not clear and closely associated with this mutation (Cooper *et al*., 1998).

4-7-3 IL-9 Polymorphism "Rs2069870" detection by polymerase chain reaction:

The study was used allelic specific PCR to identify types of polymorphism .The subjects enrolled in present study were classified into three genotypes; one homozygous for the A allele (AA) wild type, heterozygous (AG) and the last was homozygous for the allele G (GG).

The results of the current study for genotype distribution of the (rs2069870) SNP exhibited a two band 337 , 337 bp for AG, and one band for each AA 337 bp, and GG 337 bp genotype for all participants Figure (4-8)patient and (4-9)control.

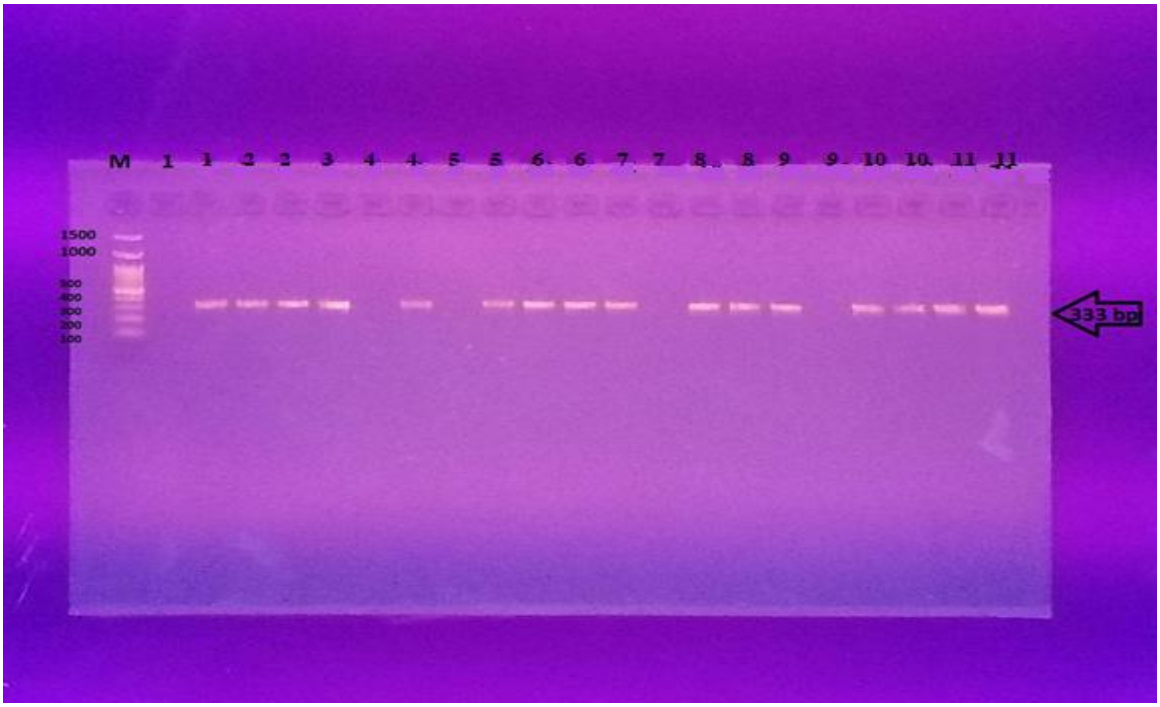


Figure (4-8) : Agarose gel electrophoresis image that showed the Allele specific-PCR product analysis of *IL-9 (rs2069870) Gene* polymorphism for patients ;

1 and 4 well represented as homozygous GG mutation alleles, 7 and 11 wells represented as wild alleles AA and 2, 3,5 8 and 10 wells represented as heterozygous alleles AG. a ladder pattern (333bp)Electrophoresis conditions, 1.5% agarose, 70V, 20mA for 1h, stained with red safe dye.

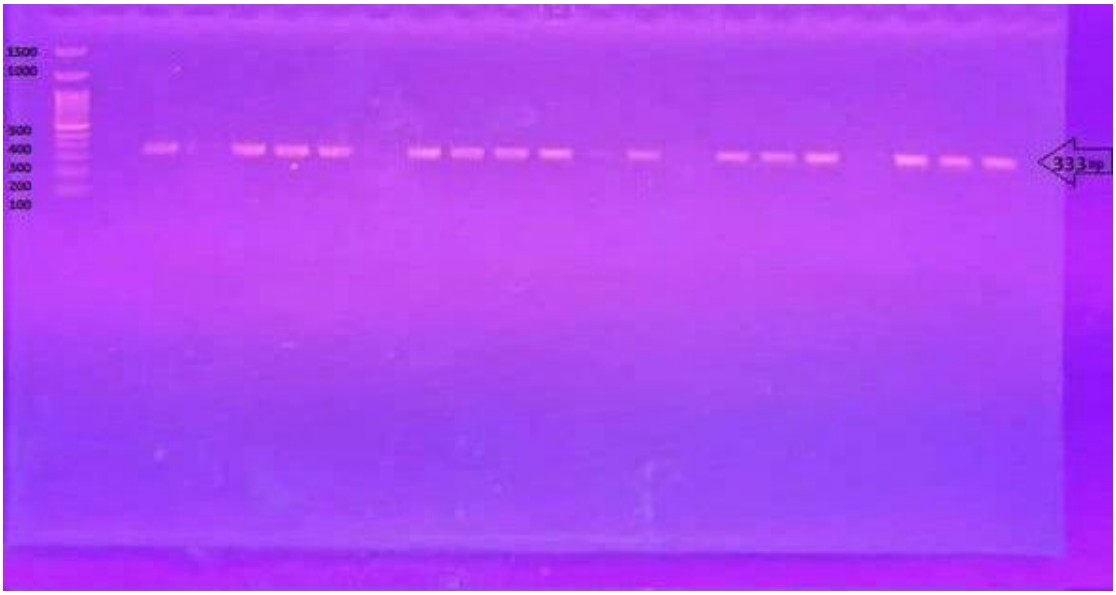


Figure (4-9) : Agarose gel electrophoresis image that showed the Allele specific-PCR product analysis of *IL-9 (rs2069870) Gene* polymorphism for control

1 ,2and 4 well represented as homozygous mutation alleles GG ,6,7,9, and 11 wells represented as wild alleles AA and 3,5,8 but 10 wells represented as heterozygous AG alleles. a ladder pattern (333bp)Electrophoresis conditions, 1.5% agarose, 70V, 20mA for 1h, stained with red safe dye.

4-7-4 Allele and genotype frequencies for rs40401 polymorphisms in the study population:

The *IL9* SNP *Rs2069870* variant were investigated in this study. All of this polymorphism, was observed in this study population Table(4-14) and were consistent with Hardy–Weinberg equilibrium, the variant alleles IL9 SNP *Rs2069870* (G allele) occurred with a lower frequency than the wild-type allele (A allele), The frequencies of the *IL9* SNP *Rs2069870* alleles were A = 0.63, G = 0.37; A relatively large frequency of the population was heterozygous (No.=46) more than wild and mutation alleles .

Table (4-14): Genotype and allelic frequency in IL-9 polymorphism among patients

Genotype Frequency Rs2069870 Patient		IL-9	HWE Frequency
Wild	AA	33	0.37
Hetero	AG	46	0.51
Homo	GG	11	0.12
Total		90	1.00
Allelic Frequency			**Frequency**
A allele		112	0.63
G allele		68	0.37
Total		180	1.00

The results of the study showed in Table (4-15) the presence of three genotypes related to IL9 SNP Rs2069870 variant in Iraqi patients and their relationship with the concentrations of the four cytokines, which include IL-3, IL-9, CD4 and CD19 in their blood; the study found significantly increased (P< 0.05) just in cytokines IL-3 in heterozygous patient, they are recorded as 15.37±2.17and it was also increased (P< 0.05) in cytokines IL-9 in homozygous patient they are recorded as 18.07±2.36 pg/ml . Otherwise, the study don't found any association significant between three genotypes with CD4 and CD8 cytokines.

Table (4-15): Concentration of cytokines among patient with IL-9 Polymorphisms

Genotype Frequency Rs2069870 Patient		IL-3	IL-9	CD4	CD19
Wild	**AA N= 33**	12.13±1.52	13.32±1.36	22.15±1.55	7.49±2.48
Hetero	**AG N= 46**	15.37±2.17	14.69±2.79	21.03±1.94	7.67±1.85
Homo	**GG N= 11**	12.17±2.31	18.07±2.36	21.8±1.55	6.46±1.16
p.value	**90**	0.0331	0.015	0.789	0.924

CD4+ helper cells release IL-9, a cytokine that promotes cell proliferation and inhibits apoptosis. Connecting this cytokine to many biological processes2 is the interleukin 9 receptor (IL9R), which acts by activating a variety of signal transducer and activator (STAT) proteins. Multiple cell types and tissues are affected by IL-9, and it is primarily involved in immune responses to parasites and the pathophysiology of allergic disorders such asthma and bronchial hyperreactivity (Parker *et al*., 2013). On the other hand, On the other hand, the study observed a significant increase in IL- 9 in Iraqi patients who have a wild allele compared to other patients who have a homozygous mutation and heterozygous alleles, Il-9 is a cytokine that is released by CD4+ helper cells that works as a regulator of a number of hematopoietic cells. The principal impacts of IL-9 are noticed in response to infections; however, the primary purpose of IL-9 is to increase the proliferation and function of mast cells. This cytokine inhibits the programmed cell death known as apoptosis while simultaneously promoting cell division (Wilhelm *et al*., 2011).

Some of studies illustrated that dichotomous role of interleukin-9 (IL-9) in different types of cancer has been elucidated in recent research. Nevertheless, the role of this particular function has been examined in cases of laryngeal squamous cell carcinoma (Pasvenskaite *et al*., 2021).

Some research has been reported that there is a relationship between tonsillitis and inflammatory process. Interleukin 9 (IL-9) is one of the cytokines that play a role in the process of inflammation and tissue damage that would affect by oral microflora (Deo & Deshmukh, 2019).

Over 700 different types of bacteria may be found in the oral cavity, making it the second most varied microbiota after the digestive tract. It provides a favorable environment for a wide variety of microorganisms, such as bacteria, fungus, viruses, and protozoa. Microorganisms are able to colonize the mouth's hard tooth surfaces and soft oral mucosa due to the mouth's diversity of habitats (Bacali *et al*., 2022).

One of the most common processes involved in the pathophysiology of a variety of diseases is inflammation. Inflammation, which is classified as a signal transduction cascade that helps identify and eliminate foreign elements and induces tissue repair, is present when there is an injury or infection (Schmid-Schönbein *et al*., 2006).

There are five different types of IL-9 polymorphism: *rs2069885* is a missense variant, which results in a change in the amino acid sequence from threonine to methionine; *rs1859430* and rs2069884 are intronic variants that are located in the coding region of *IL-9*; *rs11741137* is a downstream gene variant; and *rs2069870* is an upstream gene variant with no known function (Vilkeviciute *et al*., 2021).On the other hand, significant correlations between IL-9 polymorphism "*rs2069870*" with

both lower LDL-c and total cholesterol levels were discovered among patient suffering from coronary artery disease in a Chinese Han population (Zha *et al*., 2022).

The study did not notice any clear significant differences in each of the four cytokines IL-3, IL-9 CD4 and CD19 in relation to healthy subjects who they have genotype frequency "rs2069870" among there chromosomes as in Table (4-15).

Table (4-15): Concentration of cytokines among control with *IL-9* polymorphisms rs2069870

Genotype Frequency Rs2069870 Control		IL-3	IL-9	CD4	CD19
Wild	**AA N= 36**	16.87±4.82	13.18±3.44	13.18±2.13	4.14±0.43
Hetero	**AG N= 6**	15.63±3.48	12.27±3.08	14.53± 2.62	3.86±0.32
Homo	**GG N= 3**	14.15±4.42	14.96±3.25	15.94±3.34	4.03±0.21
P. value	**45**	0.581	0.438	0.252	0.187

The table (4-15)show the 45 samples of healthy control wild AA increased in IL3 and the Hetero AG increase in IL3,CD4 and homo GG increase in IL3,IL9 and CD4

Conclusions and Recommendations

5-1 Conclusions:

1- Tonsillitis is a common infection in both children and adults.

2- *Streptococcus pyogenes* and *Staphylococcus aureus* are the most causative Gram positive bacteria agent for tonsillitis .

3-*Klebsiella pneumonia* Gram negative bacteria isolates from tonsillitis showed the most commonly in the patients .

4-When estimation the concentration of systematic immunity (serum) of all parameters by ELISA test CD4,CD19,IL9 higher in patient than control but IL3 higher in control than patients .

5-When estimation the concentration of Locally immunity (swab) of all parameters by ELISA test CD4,CD19,IL9 higher in patient than control but IL3 higher in control than patients .

6- Regarding *IL3 (rs40401)* genotypes the heterozygous genotype C/T and the homozygous genotype TT and allele AT were non-significant risk factor for tonsillitis, whereas, genotype CC was a non-significant protective factors against tonsillitis.

7- Regarding of *IL-9 (rs2069870)* genotypes the heterozygous genotype A/G and the homozygous genotype GG and allele AA were non-significant risk factor for tonsillitis, whereas, genotype GG was a non-significant protective factors against tonsillitis.

5-2 Recommendations :

1- Used different methods to diagnosis of the causative agent of tonsillitis by another molecular method.

2- Study the effect of antibiotic among the patient with tonsillitis infection.

3- Further studies of the another interleukins that effect of this disease local and systematic immunity .

4- Study the effect of genes CD4 &CD19 by allele specific method

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الخلاصة

التهاب اللوزتين هو مرض شائع ومنتشر والتهاب اللوزتين في البلعوم. يمكن أن يغزو التهاب اللوزتين العديد من الكائنات الحية، بما في ذلك البكتيريا والفيروسات والعث والفطريات والطفيليات. قامت الدراسة الحالية بتقييم بعض المؤشرات الواضحة والخلوية على المستويين الجهازي والمحلي لعدد مرضى التهاب اللوزتين الحاد. التهاب اللوزتين المزمن والتكرار الناتج عن البكتيريا سالبة الجرام وسالبة الجرام جاء من أذان إلى أندرويد 2022. وقد درس 90 مريضاً منهم 51 ذكراً و39 أنثى، زاروا مستشفى الإمام الحسن المجتبى (عليه السلام). تعاني العيادات التعليمية وبعض العيادات الخارجية في كربلاء من التهاب اللوزتين. وتم تشخيصهم عن طريق الأذن والأنف والحنجرة وتم تصنيفهم. كان 45 فردًا من نفس الألعاب مجموعة مراقبة صحية. بلغ عدد الذكور 28 وعدد الإناث 17. وكان جميع أفراد العينة في الفئة العمرية ما بين (1-60) سنة.

جميع المرضى والأصحاء، تم أخذ عينة دم (مع وبدون مضادات التخثر) للدراسة المناعية والجزيئية. وكذلك مسحتين من اللوزتين لكل مريض، توضع إحداهما في محلول ملحي معقم لغرض دراسة المناعة الموضعية، والأخرى توضع في محلول يحتوي على وسط زرعي للزراعة الميكروبية.

تم تشخيص البكتيريا المأخوذة من اللوزتين وصبغها بصبغة جرام لتحديد ما إذا كانت إيجابية أم سلبية بعد زراعتها على مجموعة من أوساط الزراعة. كما تم تأكيد التشخيص بواسطة طريقة فيتيك لتحديد نوع البكتيريا الشائعة في المرض.أكثر البكتيريا إيجابية الجرام شيوعًا هي90/24(26.6%) Streptococcus pyogenes بينما كانت البكتيريا سالبة الجرام الأكثر شيوعًا هي90/11(12.2%) Klebsiella pneumonia). يمكن تصنيف نوع العدوى إلى حاد 90\48 (53.3%). المزمنة 90/37 (41.1%) والمتكررة 90/5 (5.6%).

تم إجراء اختبار مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد التراكيز لجميع العينات بعد أخذ عينتين لكل شخص، إحداهما موضعية وتتكون من مسحات يتم مسحها من اللوزتين ووضعها في محلول ملحي، والثانية كان نظاميًا، والذي كان مصلًا. وبعد وضع عينة الدم في مضاد التخثر واستخدام جهاز الطرد المركزي، كانت النتيجة زيادة مستويات CD4 وCD19 وIL9. زاد التركيز في المرضى والأصحاء محلياً وجهازياً، حيث بلغ تركيز CD4 في المرضى (22.11±3.66) بيكوغرام/م و (17.39±1.38) بيكوغرام/م في الأشخاص

الأصحاء جهازياً، وكان التركيز في المرضى (21.97 ± 1.53) بيكوغرام / م و (15.76 ± 1.97) بيكوغرام / م في الأشخاص الأصحاء موضعياً، في حين أن تركيز CD19 في المرضى (49.34 ± 3.57) نانوغرام / م و (22.81 ± 6.59) نانوغرام / م في الأشخاص الأصحاء بشكل جهازي ، كان تركيز IL9 في المرضى (47.33 ± 2.91) نانوغرام / م و (25.9 ± 3.83) نانوغرام / م في الأشخاص الأصحاء محلياً، بينما كان تركيز IL9 في المرضى (13.07 ± 4.92) بيكوغرام / م و (10.74 ± 4.66). بيكوغرام/م في الأشخاص الأصحاء بشكل جهازي، كان التركيز في المرضى (12.91 ± 3.35) بيكوغرام/م و (10.18 ± 3.94) بيكوغرام/م في الأشخاص الأصحاء موضعياً، على عكس IL3، الذي انخفض في المرضى وزاد محلياً في الأشخاص الأصحاء. وجهازياً، حيث كان تركيز IL3 في المرضى (9.119 ± 2.66) بيكوغرام/م و (11.31 ± 2.98) بيكوغرام/م في الأشخاص الأصحاء. من الناحية الجهازية، يبلغ التركيز لدى المرضى (5.69 ± 1.107) بيكوغرام/م و (8.76 ± 2.181) بيكوغرام/م عند الأشخاص الأصحاء موضعياً.

تمت دراسة تعدد الأشكال الجينية لجميع المرضى باستخدام الطريقة الخاصة بالأليل باستخدام البادئ الخاص بالجين، وتم العمل على جينين، الأنماط الجينية والأليلات IL-3 rs40401). أول وضع للسيادة المشتركة في الوراثة، كان هناك تباين كبير في توزيع النمط الوراثي لـ CC السائد بطريقة تجعل توزيع النمط الوراثي المتغاير CT أكثر تواتراً في مجموعة المرضى ولكن النمط الوراثي المتماثل TT ليس له تباين كبير في توزيع النمط الوراثي، IL-9 rs2069870). الأنماط الجينية والأليلات. أول وضع للهيمنة المشتركة في الميراث، كان هناك تباين كبير في توزيع النمط الوراثي للـ AA السائد بطريقة تجعل توزيع النمط الجيني المتغاير AG أكثر تواتراً في مجموعة المرضى ولكن النمط الجيني المتماثل GG ليس له تباين كبير في توزيع النمط الجيني.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل - كلية العلوم
قسم علوم الحياة

تقييم بعض المؤشرات المناعية والجزيئية في مرضى اصابات التهاب اللوزتين البكتيرية

اطروحة مقدمة
الى مجلس كلية العلوم/ جامعة بابل
وهي جزء من متطلبات نيل درجة فلسفة الدكتوراه
في علوم الحياة

من قبل

زينه علي حسين علي
(بكلوريوس علوم بنات/ جامعة بابل ٢٠١٦)
(ماجستير علوم بنات/ جامعة بابل 2019)

بإشراف

أ.د ازهار عمران الذهب

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