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Assessment of ASO Titer in Post Tonsillectomy and Those Without Throat Infection in Yemeni People, Sana'a City, Yemen 2022-2023

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بسم الله الرحمن الرحيم

" قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم "

صدق الله العظيم

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Dedication

Hearts filled with the light of love and loyalty and covered with the scent of musk, crowned with roses and narcissus, and the contentment of this humble look, to those who planted in our hearts the love of knowledge.

"Our fathers may God protect them"

To whom to carry us here on weakness

"Our dear mothers"

To whom we stress our roses and our hearts rejoice in them.

"Our brothers"

To those who helped us acquire knowledge.

"Our teachers"

And to students of knowledge, we dedicate this research.

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Abstract

Background: Serum Antistreptolysin O (ASO) titer is raised when there is an infection of any organ of the body, by Group A beta-hemolytic streptococci (GABHS), Group C or Group G streptococci, increased serum ASO titer should not be the only deciding criterion for tonsillectomy. Therefore, we aimed to the assessment of ASO titers in post-tonsillectomy and those without throat infection in Yemeni people, Sana'a, Yemen.

Materials and Methods A cross-sectional study was done on 104 samples divided into two groups. Group I included 52 People post-tonsillectomy and group II included 52 people without tonsillitis or pharyngitis. Analysis of data was done by using the statistical package for social science (SPSS version 26).

Results: A total of 104 samples including 57 males and 47 females were tested for ASO serum levels.48 were positive and 56 were negative. Of 48 positive cases, 21 were male and 27 were female. The positive ASOT readings for the people without tonsillitis or pharyngitis group and the people post tonsillectomy group were 26 (50.0%) and 22 (42.3%), respectively.

Conclusion: In this study, ASO antibodies were high in people without tonsillitis or pharyngitis and in people post tonsillectomy, ASO antibodies in our study were found in in Females showed more positivity compared to males, ASO positivity was seen in the 11–20 years of age group, there is an association between the increase serum level of ASO with a skin infection in People without tonsillitis or pharyngitis and there is no effect of use long of Benzathine Penicillin on serum level of ASO.

Keywords: Antistreptolysin O titer, *Streptococcus pyogenes*, tonsillectomy, Yemen

List of Abbreviations

ASO	Anti streptolysin O
AGN	Acute glomerulonephritis
CD	Cluster of differentiation
GABHS	Group A beta hemolytic streptococci
GAS	Group A streptococci
LAMP	loop-mediated isothermal amplification
MALDI-TOF MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry
PCR	polymerase chain reaction
PYR	Pyrrolidonyl Arylamidase
RF	rheumatic fever
SDB	sleep-disordered breathing
SLO	Streptolysin O
SLS	Streptolysin S
SPSS	Statistical Package For Social Science

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Chapter One

Introduction

1.1. Introduction:

A raised ASO titer level is one of the most relevant retrospective serological indices of antecedent GABHS infection. A single titer of more than 200 iu/ml is considered a raised value. Serum ASO titer is raised when there is an infection of any organ of the body by GABHS, so the increased serum ASO titer should not be the only deciding criterion for tonsillectomy if GABHS is not present in the palatine tonsils. GABHS is the most common bacteria that cause acute tonsillitis. Streptococcal infection can lead to rheumatic fever. The incidence of rheumatic fever in untreated cases of tonsillitis is 3% and in treated cases, the incidence falls to 0.3%. It has been estimated that rheumatic heart disease constitutes 25% to 40% of all cardiovascular diseases in third-world countries. Recurrent tonsillitis is the most common indication of tonsillectomy.**(El-magd & Meguid, 2016)**

The ASO titer is the most widely used and the best standardized serological test of a recent streptococcal infection. A single titer of more than 200 iu/ml is considered a raised value. Classical descriptions suggest that the ASO titer rises a week following infection and peaks at 3 to 5 weeks, begins to fall at 6 weeks, returning to pre-infection levels at around 8 months, a longitudinal study of 160 children closely followed over a 2- year period suggests that the waning in ASO titer is frequently prolonged; two-thirds of children maintained titers above pre infection levels for more than 1 year. **(Viswanathan, Nair, & Thulseedharan, 2000)**

The genus Streptococcus consists of Gram-positive, aerobic bacteria, which appear as chains the under microscope. The organisms in this genus are characterized by a coccus appearance, a thick cell wall, and aerobic utilization of glucose. Over many years, the classification of Streptococci into major categories has been based on a series of observations: (1) colony morphology and hemolytic reaction on blood agar; (2) serologic specificity of the cell wall group-specific substance (Lancefield Classification) and other cell wall or capsular antigens; (3) biochemical reaction and resistance to physical and chemical factors; and (4) ecological feature. Additional biochemical tests and molecular genetics also have been used to study the relationships of streptococcal species to each other. **(Hassan, 2005)**

1.2. Objectives:

1.2.1. General objective:

To assess the ASO Titer in Post Tonsillectomy and Those Without Throat Infection in Yemeni People, Sana'a City. 2022.

1.2.1. Specific objectives:

1. To assess the ASO Titer in Post Tonsillectomy People.
2. To assess the ASO Titer in People Without Throat Infection.
3. To compare the relationship between ASO titer in patients after tonsillectomy and people without tonsillitis or pharyngitis.
4. To assess if there are medical conditions that lead to increase ASO titer.
5. To compare the level of ASO titer in those people used Benzathine Penicillin and in not used.

1.3. Research Hypothesis:

ASO titer in people without throat infection and post tonsillectomy will be normal or negative.

Chapter Two

Literature Review

2.1. Classification of Streptococci:

The classification of streptococci into major categories has been based on a series of observations over many years: (1) colony morphology and hemolytic reactions on blood agar, (2) serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens, (3) biochemical reactions and resistance to physical and chemical factors, and (4) ecologic features. More recently, molecular genetics has replaced phenotypic methods in the taxonomic assignment of these organisms.

A. Hemolysis:

Many streptococci can hemolyze red blood cells in vitro in varying degrees. Complete disruption of erythrocytes with the clearing of the blood around the bacterial growth is called β-hemolysis. Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α-hemolysis. Other streptococci are nonhemolytic (sometimes called γ- hemolysis).

Clinically important streptococci are traditionally differentiated based on their hemolysis pattern, that is, β-hemolytic vs non-hemolytic streptococci. Beta-hemolytic streptococci are also referred to as pyogenic streptococci, which include the human-pathogenic species *S. pyogenes, S. agalactiae, and S. dysgalactiae*. The classification of hemolytic patterns is used primarily with the streptococci although with other bacteria because the disease may also typically produce a variety of hemolysis.**(Riedel, Morse, Mietzner, & Miller, 2019)**

B. Group-Specific Substance (Lancefield Classification):

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups A–H and K–U. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar. For group Streptococci, this is rhamnose-N-acetylglucosamine; for group B, it is a rhamnose-glucosamine polysaccharide; for group C, it is rhamnose-N-acetylgalactosamine; for group D, it is glycerol teichoic acid containing d-alanine and glucose; and for group F, it is glucopyranosyl-N-acetylgalactosamine. **(Riedel et al., 2019)**

Extracts of group-specific antigens for grouping streptococci are prepared by a variety of methods, including extraction of centrifuged culture treated with hot hydrochloric acid, nitrous acid, or form amide; by enzymatic lysis of streptococcal cells (eg, with

pepsin or trypsin); or by autoclaving of cell suspensions. These extracts contain the carbohydrate group-specific substance that yields precipitin reactions specific antisera. This permits the arrangement of many streptococci into groups A–H and K–U. Typing is generally done only for groups A, B, C, F, and G, which cause disease in humans and for which reagents are available that allow typing using simple agglutination or color reactions.**(Riedel et al., 2019)**

C. Capsular Polysaccharides:

The antigenic specificity of the capsular polysaccharides is used to classify S. pneumoniae into more than 90 types and to type the group B streptococci (S. agalactiae).

D. Biochemical Reactions:

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents. Biochemical tests are most often used to classify streptococci after colony growth and hemolytic characteristics have been observed.**(Riedel et al., 2019)**

Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G. For example, the viridians streptococci are α-hemolytic or nonhemolytic and do not react with the antibodies commonly used for the Lancefield classification. Speciation of the viridians streptococci requires a battery of biochemical tests (see Table 14-1). However, because biochemical reactions are labor intensive and often unreliable, laboratories with molecular capabilities, such as gene sequencing or that have implemented mass spectrometry for organism identification (matrix-assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS]), are replacing phenotypic tests with these methods when the identification of viridians streptococci is required.**(Riedel et al., 2019)**

2.2. *Streptococcus pyogenes* (Group A streptococcus)

Streptococcus pyogenes is a gram-positive, extracellular, non-motile, β-hemolytic bacterial pathogen that belongs to Group A streptococci, 0.6-1.0 micrometer in diameter. Streptococci divide in one plane and thus occur in pairs or chains of varying

lengths, especially in liquid media and clinical specimens. The metabolism of *S. pyogenes* is fermentative; the organism is a catalase-negative aerotolerant anaerobe (facultative anaerobe) and requires an enriched medium containing blood to grow. **(Subhash, 2012)**

It is capable of causing a wide variety of diseases globally with humans being their only known natural biological host. There is no environmental reservoir known for Group A streptococci and transmission of the bacteria occurs almost always from person to person. The primary sites for colonization involve the nasal and oropharyngeal mucosal epithelium of the upper respiratory tract as well as the superficial layers of the epidermis where GAS can undergo successful reproductive growth and transmission to the next host. Two reasons why this bacterium presents such a challenge for the human body are the existence of more than 200 distinct serotypes and the enormous repertoire of different virulence factors. The ingenious way the bacterium counteracts host defenses largely contributes to the success of GAS in establishing infections in its human host. **(Fritzer, 2010)**

2.3. Extracellular Surface Molecules and Virulence Factors.

Hyaluronic acid capsule:

The group A streptococcal capsule is composed of a polymer of hyaluronic acid containing repeating units of glucuronic acid and Nacetylglucosamine. The hyaluronic acid capsule is required for resistance to phagocytosis. Acapsular mutant strains were altered in their virulence and colonization capacities in animal models. In addition, the capsule may be an important adherence factor in the pharynx, since it binds CD44 on epithelial cells Epidemiologic evidence linking highly mucoid strains with rheumatic fever and severe invasive streptococcal disease suggests that the capsule could play an important role in invasive infections in humans. The fact that the capsule is antiphagocytic and promotes resistance to phagocytosis is supported by data demonstrating that hyaluronidase treatment of encapsulated streptococci increases their susceptibility to phagocytosis. However, anti-capsule antibody is not opsonic and does not protect against infection by neutralizing the antiphagocytic effects of the capsule. More recent work provides definitive evidence that the capsule is a major virulence determinant involved in resistance to phagocytosis. **(Hassan, 2005)**

M protein.

This substance is a major virulence factor of *S. pyogenes*. M protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall. When the M protein is present, the streptococci are virulent, and in the absence of M-type-specific antibodies, they can resist phagocytosis by polymorphonuclear leukocytes by inhibiting the activation of the alternate complement pathway. *S. pyogenes* that lack M protein is not virulent. Immunity to infection with group A streptococcus is related to the presence of type-specific antibodies to the M protein. Because there are more than 150 types of M protein, a person can have repeated infections with *S. pyogenes* of different M types. Both groups C and G streptococci have genes homologous to the genes for the M protein of group A, and M proteins similar to those of group A have been found in groups C and G streptococci. **(Riedel et al., 2019)**

The M protein molecule has a rod-like coiled structure that separates functional domains. The structure allows for a large number of sequence changes while maintaining the function, and the M protein immune determinants, therefore, can readily change. There are two major structural classes of M protein, classes I and II. It appears that the M protein and perhaps other streptococcal cell wall antigens have an important role in the pathogenesis of rheumatic fever. Purified streptococcal cell wall membranes induce antibodies that react with human cardiac sarcolemma; the characteristics of the cross-reactive antigens are not clear. A component of the cell wall of selected M types induces antibodies that react with cardiac muscle tissue. Conserved antigenic domains on the class I M protein cross-react with human cardiac muscle, and the class I M protein may be a virulence determinant for rheumatic fever.**(Riedel et al.,** 2019)

Lipoteichoic acid.

In *Streptococcus pyogenes*, lipoteichoic acid is associated with the M protein that protrudes from the cell membrane through the peptidoglycan layer. The long molecular M protein together with the lipoteichoic acid from microfibrils facilitates the attachment of *S pyogenes* to animal cells**.**

Toxins and Enzymes.

More than 20 extracellular products that are antigenic are elaborated by *S. pyogenes,* including the following.

A. Streptokinase

This enzyme is produced by *S. pyogenes* as well as by group C and G streptococci. Two types of streptokinase have been described—streptokinase A and B. Streptokinase activates plasminogen to form plasmin, which breaks down the fibrin barrier around the infected site, thereby facilitating the spread of the infection. This thrombolytic property is made good use of in the medical management of myocardial infarction. Antibodies appear against streptokinase (A and B) during infection and are diagnostic. **(Subhash, 2012)**

B. Deoxyribonucleases.

Four types of deoxyribonucleases have been described—deoxyribonucleases A, B, C, and D. Most strains of *S. pyogenes* produce these enzymes. The enzyme depolymerizes free DNA present in the pus, thereby reducing the viscosity of pus and helping in the spread of the infection. The enzymes are antigenic, and the demonstration of anti-deoxyribonuclease B antibody in serum is diagnostic of *S. pyogenes* infections, particularly of skin infections. **(Subhash, 2012)**

C. Hyaluronidase.

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source. After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.**(Riedel et al., 2019)**

D. Pyrogenic Exotoxins (Erythrogenic Toxin).

Pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins (Spe): A, B, and C. SpeA has been the most widely studied. It is produced by group A streptococcus which carries a lysogenic phage. Streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever. Most strains of group A streptococci isolated from

patients with streptococcal toxic shock syndrome either produce Spe A or have the gene that codes for it; in contrast, only about 15% of group A streptococci isolated from other patients have the gene. Spe C, also encoded by a phage, may contribute to the syndrome. Spe B, a potent protease, interferes with phagocytosis. The group A streptococci associated with toxic shock syndrome are primarily of M protein types 1 and 3.**(Riedel et al., 2019)**

The pyrogenic exotoxins act as superantigens, which stimulate T cells by binding to the class II major histocompatibility complex in the Vβ region of the T-cell receptor. The activated T cells release cytokines that mediate shock and tissue injury. The mechanisms of action appear to be similar to those caused by staphylococcal toxic shock syndrome toxin-1 and the staphylococcal enterotoxins.**(Riedel et al., 2019)**

E. Hemolysins.

Two types of hemolysins, oxygen-labile streptolysin O (SLO) and oxygen-stable and serum-soluble streptolysin S (SLS) are produced by S. pyogenes.

1. Streptolysin O.

SLO is an oxygen-labile and heat-labile protein with a molecular weight of 50,000–75,000 Da. It causes beta hemolysis only when colonies are grown under the surface of the blood agar plate. It is antigenic, and antibodies (ASLO) against it develop in group A streptococcal infection. Demonstration of ASLO antibodies is important for the determination of a recent group A streptococcal infection and also the late complications of streptococcal infections after the organisms have been eliminated from the host. The SLO cross-reacts with similar hemolysins produced by streptococci of groups C and G, pneumolysins of *S. pneumoniae*, tetanolysin of *Clostridium tetani,* theta toxin of *Clostridium perfringens*, aerolysin of *Bacillus cereus*, and listeriolysin of *Listeria monocytogenes*. **(Subhash, 2012)**

2. Streptolysin S.

SLS is a serum-soluble (hence named S) and oxygen-stable protein. It is a small polypeptide of 20,000 Da and is nonantigenic. Hence, no antibodies against this toxin are demonstrated in serum. This toxin Is responsible for hemolysis around the colonies grown on the surface of the blood agar and inhibits chemotaxis and is antiphagocytic. **(Subhash, 2012)**

2.4. Pathogenesis and Clinical Findings:

A variety of distinct disease processes are associated with *S pyogenes* infections. The infections can be divided into several categories.

A. Diseases Attributable to Invasion by *S pyogenes*, β-Hemolytic Group A Streptococci.

The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream. **(Brooks et al., 2007)**

Erysipelas— If the portal of entry is the skin, erysipelas results, in massive brawny edema and a rapidly advancing margin of infection.

Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions. Pain, tenderness, swelling, and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings: In cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.

Necrotizing fasciitis (streptococcal gangrene)— There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia. Bacteria other than *S pyogenes* can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fasciitis have sometimes been termed flesh-eating bacteria. **(Brooks et al., 2007)**

Puerperal fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially septicemia originating in the infected wound (endometritis**). (Brooks et al., 2007)**

Bacteremia or sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal. *S pyogenes* bacteremia can also occur with skin infections, such as cellulitis and rarely pharyngitis. **(Brooks et al., 2007)**

B. Diseases Attributable to Local Infection with *S pyogenes* and Their Byproducts.

Streptococcal sore throat—The most common infection caused by β-hemolytic *S pyogenes* is a streptococcal sore throat or pharyngitis. *S pyogenes* adhere to the pharyngeal epithelium using lipoteichoic acid–covered surface pili and using hyaluronic acid in encapsulated strains. The glycoprotein fibronectin (MW, 440,000) on epithelial cells probably serves as a lipoteichoic acid ligand. In infants and small children, the sore throat occurs as a subacute nasopharyngitis with a thin serous discharge and little fever but with a tendency of the infection to extend to the middle ear and the mastoid. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate; enlarged, tender cervical lymph nodes; and (usually) a high fever. Twenty percent of infections are asymptomatic**. (Brooks et al., 2007)**

A similar clinical picture can occur with infectious mononucleosis, diphtheria, gonococcal infection, and adenovirus infection. *S pyogenes* infection of the upper respiratory tract does not usually involve the lungs. Pneumonia, when it does occur, is rapidly progressive and severe and is most commonly a sequela to viral infections, such as influenza or measles, which seem to greatly enhance the predisposition to bacterial superinfection with this and other pathogens, such as *S pneumonia.* **(Brooks et al., 2007)**

Streptococcal pyoderma—Local infection of superficial layers of skin, especially in children, is called impetigo. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted. It spreads by continuity and is highly communicable, especially in hot, humid climates. More widespread infection occurs in eczematous or wounded skin or in burns and may progress to cellulitis. Group A streptococcal skin infections are often attributable to M types 49, 57, and 59–61 and may precede glomerulonephritis (GN) but do not lead to rheumatic fever. A clinically identical infection can be caused by Staphylococcus aureus and sometimes both *S pyogenes* and *S aureus* are present. **(Brooks et al., 2007)**

C. Invasive Group A Streptococcal Infections, Streptococcal Toxic Shock Syndrome, and Scarlet Fever:

Fulminant, invasive *S pyogenes* infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients. The infections tend to occur after minor trauma in otherwise healthy persons with several presentations of soft tissue infection. These include necrotizing fasciitis, myositis, and infections at other soft tissue sites; bacteremia occurs frequently. In some patients, particularly those infected with group A streptococci of M types 1 or 3, the disease presents with focal soft tissue infection accompanied by fever and rapidly progressive shock with multiorgan failure. Erythema and desquamation may occur. The *S pyogenes* of the M types 1 and 3 (and types 12 and 28) that make pyrogenic exotoxin A or B are associated with the severe infections. **(Brooks et al., 2007)**

Pyrogenic exotoxins A–C also cause scarlet fever in association with *S pyogenes* pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities. Streptococcal toxic shock syndrome and scarlet fever are clinically overlapping diseases.**(Viswanathan et al., 2000)**

D. Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

After an acute *S pyogenes* infection, there is a latent period of 1–4 weeks, after which nephritis or rheumatic fever occasionally develops. The latent period suggests that these poststreptococcal diseases are not attributable to the direct effect of disseminated bacteria but instead represent a hypersensitivity response. Nephritis is more commonly preceded by infection of the skin; rheumatic fever is more commonly preceded by infection of the respiratory tract**. (Brooks et al., 2007)**

Acute glomerulonephritis—This sometimes develops 1–4 weeks after *S pyogenes* skin infection (pyoderma, impetigo) or pharyngitis. Some strains are particularly nephritogenic, principally with M types 2, 42, 49, 56, 57, and 60 (skin). Other nephritogenic M types associated with throat infections and glomerulonephritis are 1, 4, 12, and 25. After random streptococcal skin infections, the incidence of nephritis is less than 0.5%.**(Brooks et al., 2007)**

Glomerulonephritis may be initiated by antigen– antibody complexes on the glomerular basement membrane. The most important antigens are thought to be SpeB and a nephritis-associated plasmin receptor. In acute nephritis, the patient has blood and protein in the urine, edema, high blood pressure, and urea nitrogen retention; serum complement levels are also low. A few patients die, some develop chronic glomerulonephritis with ultimate kidney failure, and the majority recover completely**.**

Rheumatic fever—This is the most serious sequela of *S pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens. **(Brooks et al., 2007)**

The onset of rheumatic fever is often preceded by *S pyogenes* pharyngitis 1–4 weeks earlier, although the infection may be mild and may not be detected. In general, however, patients with more severe streptococcal sore throats have a greater chance of developing rheumatic fever. Rheumatic fever is not associated with cutaneous streptococcal infections. In the 1950s, untreated streptococcal infections were followed by rheumatic fever in up to 3% of military personnel and 0.3% of civilian children. Rheumatic fever is now relatively rare in the United States (<0.05% of streptococcal infections), but it occurs up to 100 times more frequently in tropical countries and is the most important cause of heart disease in young people in developing countries**. (Brooks et al., 2007)**

Typical symptoms and signs of rheumatic fever include fever, malaise, a migratory nonsuppurative polyarthritis, and evidence of inflammation of all parts of the heart (endocardium, myocardium, and pericardium). The carditis characteristically leads to thickened and deformed valves and to small perivascular granulomas in the myocardium (Aschoff bodies) that are finally replaced by scar tissue. Erythrocyte sedimentation rates, serum transaminase levels, electrocardiograms, and other tests are used to estimate rheumatic activity**. (Brooks et al., 2007)**

Whereas rheumatic fever has a marked tendency to be reactivated by recurrent streptococcal infections, nephritis does not. The first attack of rheumatic fever usually produces only slight cardiac damage, which, however, increases with each subsequent attack. It is therefore important to protect such patients from recurrent *S pyogenes* infections by prophylactic penicillin administration.**(Brooks et al., 2007)**

2.5. Diagnosis

2.5.1. Culturing techniques

Streptococci are generally grown on agar media supplemented with blood. This technique allows the detection of β-hemolysis, which is important for subsequent identification steps, and enhances the growth of streptococci by the addition of an external source of catalase. Selective media for culturing Gram-positive bacteria (such as agar media that contains phenyl ethyl alcohol, or Columbia agar with colistin and nalidixic acid) also provide adequate culturing conditions for *S. pyogenes.* Optimal incubation conditions for the vast majority of streptococcal strains include a temperature range of 35°C to 37°C in the presence of 5% CO2 or under anaerobic conditions. These conditions are optimized for culturing streptococcal species that belong to the viridans group, but they may not be ideal for growing *S. pyogenes*.**(Spellerberg & Brandt, 2022)**

To identify *S. pyogenes* in clinical samples, blood agar plates are screened for the presence of β-hemolytic colonies. The typical appearance of *S. pyogenes* colonies after 24 hours of incubation at 35-37°C is dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish color and have a diameter of ≥ 0.5 mm, and are surrounded by a zone of β-hemolysis that is often two to four times as large as the colony diameter. Microscopically, *S. pyogenes* appears as Gram-positive cocci, arranged in chains**.(Spellerberg & Brandt, 2022)**

2.5.2.PYR test

Pyrrolidonyl Arylamidase (PYR) testing is a rapid test used to identify group A beta-hemolytic streptococci and enterococci based on the activity of the enzyme pyrolidonyl arylamidase. The PYR test is a rapid colorimetric method that is frequently used to differentiate *S. pyogenes* from other hemolytic streptococci with similar morphology

(such as *S. dysgalactiae* subsp. *equismilis*) and looks for the presence of the enzyme pyrrolidonyl amino peptidase. Within a few minutes, the test can be performed on paper strips containing dried chromogenic substrates for pyrrolidonyl aminopeptidase. PYR positive β-hemolytic streptococci that display the typical morphology of *S. pyogenes* can be presumptively identified as *S. pyogenes*. To avoid false positive reactions caused by other PYR positive bacterial species, which may be present in mixed cultures, this test should only be performed on pure cultures. The PYR test has been found to be very simple to use and thus may be regarded as a rapid, reliable, and cost-effective method for presumptive identification of group A streptococci and enterococci in the clinical laboratory.**(Jawetz, 2013)**

2.5.3. Bacitracin susceptibility

Bacitracin test are used to differentiate *Streptococcus pyogenes* Bacteria from other nongroup A β-hemolytic streptococcus by their increased sensitivity to bacitracin. The bacitracin test, along with the Lancefield antigen A test, is used for greater specificity in the identification of *S. pyogenes,* since other β-hemolytic strains of streptococci that may contain the group A antigen are resistant to bacitracin. The bacitracin test is also used to distinguish *S. pyogenes* from other β hemolytic streptococci that are PYR-positive, such as *S. iniae and S. porcinus.* The strain being tested is streaked with several individual colonies of a pure culture on a sheep blood agar plate and a disk containing 0.04 U of bacitracin is placed on the SBA plate. After overnight incubation at 35°C in 5% CO2, a zone of inhibition surrounding the disc indicates the susceptibility of the strain.**(Jawetz, 2013)**

2.5.4. Nucleic acid detection techniques

Several years ago, a method based on nucleic acid detection was first introduced for direct *S. pyogenes* diagnosis from clinical throat swabs. The GAS Direct test identifies specific rRNA sequences of *S. pyogenes* in pharyngeal specimens by a single-stranded chemiluminescent nucleic acid probe. The test has performed well in comparison to standard streptococcal culture methods and has received FDA clearance. Sensitivity and specificity ranged from 89%–95% and 98%–100%, respectively, as compared to culture results, which reached a sensitivity of 98%–99. The GAS Direct test can be applied for primary testing, has also been used as a backup test to negative antigen tests, and is suitable for batch screening of throat cultures**. (Spellerberg & Brandt, 2022)**

A commercial polymerase chain reaction (PCR) method for the direct detection of *S. pyogenes* using the illumi gene system has recently received FDA clearance. Excellent sensitivity (99%) and specificity (99.6%) were demonstrated for the illumigene test in a multicenter evaluation study. This test relies on loop-mediated isothermal amplification (LAMP) technology with *S. pyogenes* specific primers. In 2015, two point of care tests for the detection of *S. pyogenes* in throat swabs using rapid automated PCR technology received FDA clearance. Both the cobas Strep A test, running on the cobas Liat platform and the Simplex Group A Strep Direct Test provide PCR results for individual samples within 20 minutes. Apart from FDA-released documents, however, scientific publications on the performance of these tests concerning *S. pyogenes* detection, are not yet publicly available.**(Spellerberg & Brandt, 2022)**

2.5.5. Serological tests

Serological tests are frequently used to help diagnose *Streptococcus pyogenes* infections, especially when non-suppurative sequelae are suspected. Antibody levels are typically measured to various combinations of the extracellular group A Streptococcus (GAS) antigens streptolysin O (SLO), DNase B, streptokinase, and hyaluronidase.**(Jawetz, 2013)**

The basis of anti-streptolysin O determination in serum is the neutralization of streptolysin O by anti-streptolysin O; the presence of residual un neutralized streptolysin O is demonstrated by the hemolysis of added erythrocytes. Only reduced (non oxidized) streptolysin O is active in the reaction. This determination is one of the best standardized serological methods and was the first to be developed for measuring antibodies to group A streptococci. An international standard of anti-streptolysin O is available from the Laboratory for Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark; laboratory standards for testing can easily be derived by repeated testing against the international standard.**(Shet & Kaplan, 2006)**

The range of "normal" values for anti-streptolysin O (as with other streptococcal antibodies) is variable and depends upon the age of the patient, geographical location, epidemiological setting and season of the year. Anti streptolysin O titers are commonly reported in Todd units which represent the highest dilution of serum showing complete inhibition of hemolysis. In the absence of specific information regarding the appropriate range of normal values calculated for each of these variables, single anti-streptolysin O titers are generally considered to be increased if they are at least 250 Todd units in

adults and at least 333 Todd units in children over 5 years of age. It should be stressed that single lower titers do not exclude the possibility of streptococcal infection, since their comparison with a "normal value" may not be valid for the reasons. It is therefore preferable to undertake acute and convalescent determinations. About 20% of infected individuals do not respond by an increase in anti-streptolysin O titer. Thus, a negative anti-streptolysin O titer alone cannot be used to rule out rheumatic fever or other streptococcal sequelae. Additional antibody tests (e.g. anti-DNase B, anti-hyaluronidase) may be required.**(Shet & Kaplan, 2006)**

Streptococcal skin infections are usually followed by feeble anti-streptolysin O responses, probably because of an inhibitory action on streptolysin O by cholesterol and a number of related skin lipids. "Falsely" elevated antistreptolysin O titers (not due to presence of antibody) may occur. Lipids present in serum (e.g. in patients suffering from hepatitis or nephritis) act in the reaction as nonspecific inhibitors of streptolysin O. Some bacterial contaminants of a serum sample may have a similar effect on the titer by splitting serum lipoproteins and liberating cholesterol. Freezing and thawing of the sera may destabilize lipoproteins which can also alter the titer. Several methods can be used to remove the interfering lipids from serum samples, for example, dextran sulfate to absorb the active lipid, and simple chloroform lipid extraction. The latter appears to be easier to perform. Attention should be paid to the proper reduction of streptolysin O, as many of the falsely increased titers can be ascribed to its oxidation before or during the test. Elevated titers not representing group A streptococcal infection may also occur because this extracellular antigen is also produced by streptococci of groups C and G.**(Shet & Kaplan, 2006)**

2.6. Treatment:

All *S. pyogenes* are uniformly susceptible to penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin-allergic patients and for patients with necrotizing fasciitis. However, resistance to macrolide antibiotics has been increasing in Europe and in the United States. Some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever. In acute streptococcal infections, however, every effort must be made to rapidly eradicate streptococci from the patient, eliminate the antigenic stimulus

(before day 8), and thus prevent poststreptococcal disease. Doses of penicillin or erythromycin that result in effective tissue levels for 10 days usually accomplish this. Antimicrobial drugs are also very useful in preventing reinfection with β-hemolytic group A streptococci in patients with rheumatic fever.**(Riedel et al., 2019)**

2.7. Prevention and Control

Chemoprophylaxis is most important in prevention of AGN or rheumatic fever. Long-term chemoprophylaxis using penicillin to prevent streptococcal infection is recommended for patients with a history of acute rheumatic fever (up to age 21 years) or rheumatic heart disease (lifelong). Antibiotic prophylaxis prevents streptococcal reinfection and further damage to the heart. The role of chemoprophylaxis for household contacts of patients with either acute streptococcal disease or nonsuppurative complications is yet to be ascertained.**(Subhash, 2012)**

2.8. Tonsillectomy:

Tonsillectomy is a surgical procedure performed wherein the tonsil with its capsule is completely removed by dissecting the per tonsillar space between the tonsil capsule and the muscular layer. Adenoidectomy is the surgical removal of adenoids and is commonly performed in combination with tonsillectomy. Tonsillectomy is most commonly performed for the presence of sleep disordered breathing (obstructive sleep apnea) and recurrent infections both of which compromise quality of life.**(Subramanyam, 2013)**

Tonsil surgery has been performed for over 3000 years. During the 19th century, when anesthesia became available, the surgical techniques became more refined and the frequency of operations performed increased. Prior to 1900, tonsillectomy was adopted, which did not remove the entire tonsillar tissue. However, during the turn of the 20th century, extracapsular tonsillectomy was introduced in order to avoid leaving tissue remnants – at this time, the 'focal infection theory' dominated clinical literature. Tonsillectomy involves the full surgical excision of the tonsils. This may be done alone or in conjunction with an excision of the adenoids (adenotonsillectomy). During the first half of the 20th century both tonsillectomy and tonsillectomy were performed, but

by 1950 only total tonsillectomy was deemed appropriate, and the primary reason for surgery was still recurrent throat infections. Many countries still undertake tonsillectomy, in particular for those on the sleep-disordered breathing (SDB) spectrum, especially among small children. Total tonsillectomy is the preferred operation for recurrent throat infections in the UK**.(Koshy, 2015)**

Tonsillectomy is the most frequently performed otolaryngological procedure, especially in young children; it is effective in reducing the number and duration of episodes of sore throat in children, the gain being more marked in those most severely affected. 9,10 However, pediatricians prefer to treat children having tonsillitis with long-acting penicillin. The recommended dose of benzathine penicillin G is 600,000U intramuscularly for patients weighing 27 kg (60lb) or less, and 1,200,000 U for patients weighing more than 27 kg.**(Bharti, Mohindroo, Mohindroo, Sharma, & Kanga, 2017)**

Chapter Three

Subjects & Methods

3. Subjects & methods:

3.1. Study design

Cross-Sectional study.

3.2. Study area and period

We collected samples at 48, Al- Kuwait,21 September University, Al- Thawra General, Al- Gumhori Teaching and University of Science and Technology Hospitals and ENT Clinics Company in Sana'a City July 2022 to December 2022.

3.3. Inclusion criteria

People post tonsillectomy and people without history of tonsillitis or pharyngitis.

3.4. Exclusion criteria

People with tonsillitis or pharyngitis and People post tonsillectomy for less than 6 months ago.

3.5. Sample size

The total number of 104 People were enrolled in this study.

They are divided in two groups, 52 People post tonsillectomy (Group one) and the other 52 people without tonsillitis or pharyngitis (Group two).

3.6. Data collection

3.6.1. A pre design questionnaire: written by Arabic language used to collect demographic information from all participant e.g. age, gender…etc.

3.6.2 Blood samples collection

3-5 ml of Blood samples were collected into plain tubes and allowed to clot. The clot samples were centrifuged immediately for 10 min at 3000 rpm.

3.7. Laboratory tests

Serum Anti-Streptolysin Antibodies by Latex method.

3.8. Statistical analysis

Data was processed and analyzed using the statistical package for Social Sciences (SPSS) version 26. Descriptive frequencies and Chi-square test was used to test the association between variables.

3.9. Ethical consideration

The study protocol was endorsed by the Deanship of Graduate Studies and Scientific Research at 21 September University of Medical and Applied Sciences, this study was explained to participants and a consent was obtaining from each participated.

Chapter Four

Result & Discussion

4. Result & Discussion:

4.1. Result

A total of 104 blood samples were collected and processed for the detection of anti-streptolysin O antibodies, of these 57 were males and 47 females. Of whom 52 were people without tonsillitis or pharyngitis and 52 were people post tonsillectomy.

Anti-streptolysin O "ASO" titer in People without tonsillitis or pharyngitis (n=52) was distributed as follows: 26 (50.0%) of subjects were "ASO" titer positive and 26 (50.0%) were "ASO" titer negative. **Table 4.1**

Table 4.1: Anti-streptolysin O "ASO" titer in People without tonsillitis or pharyngitis.

Parameter		People without tonsillitis or pharyngitis N(%) No=52
ASO Titer	**Positive (>200 IU/ml)**	26 (50.0%)
	Negative (≤200 IU/ml)	26 (50.0%)

Anti-streptolysin O "ASO" titer in people post tonsillectomy (n=52) was distributed as follows: 22 (42.3%) of subjects were "ASO" titer positive and 30 (57.7%) were "ASO" titer negative. **Table 4.2**

Table 4.2: Anti-streptolysin O "ASO" titer in people post tonsillectomy.

Parameter		People Post Tonsillectomy N (%) No=52
ASO Titer	**Positive (>200 IU/ml)**	22 (42.3%)
	Negative (≤200 IU/ml)	30 (57.7%)

ASO titer between people without tonsillitis or pharyngitis and people post tonsillectomy. The results showed that serum anti-streptolysin O Antibodies was non-significant (P value>0.05) when compared with People without tonsillitis or pharyngitis**. Table 4.3**

Table 4.3. Comparison ASO titer between People without tonsillitis or pharyngitis and People Post Tonsillectomy.

Parameter		People without tonsillitis or pharyngitis N(%) No=52	People Post Tonsillectomy N (%) No=52	*P* .value
ASO Titer	**Positive (>200 IU/ml)**	26 (50.0%)	22 (42.3%)	0.431
	Negative (≤200 IU/ml)	26 (50.0%)	30 (57.7%)	

The relation between Anti-streptolysin O "ASO" titer category and gender in studied sample. There was statistically significant relation between Anti-streptolysin O "ASO" titer category and gender in studied sample (P < 0.05**). Table 4.4**

Table 4.4: Relation between Antistreptolysin O "ASO" titer category and gender in studied sample.

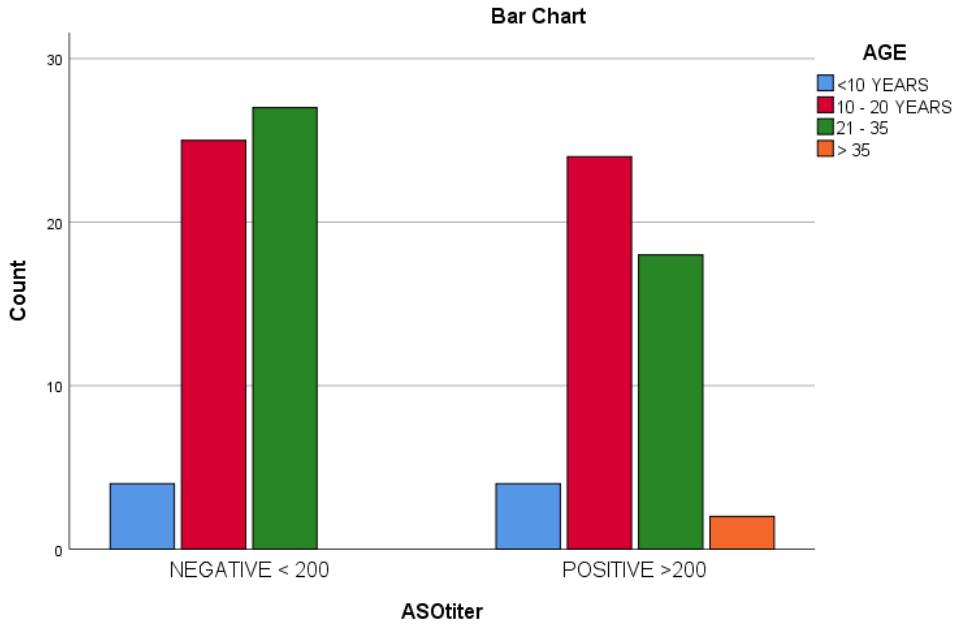
Gender	ASO **Titer (N=102)**		***P .value***
	Positive(>200 IU/ml)	**Negative((**≤200 IU/ml**)**	
Male	21 (36.8%)	36 (63.2%)	**0.036**
Female	27(57.4%)	20(42.6%)	

The relation between Anti-streptolysin O "ASO" titer category and age in studied sample. There was no statistically significant relation between Anti- streptolysin O "ASO" titer category and age in studied group (P > 0.05). **Table 4.5**

Table 4.5: Relation between Antistreptolysin O "ASO" titer category and age in studied sample.

Age (years)	ASO **Titer (N=102)**		***P .value***
	Positive(>200 IU/ml)	**Negative (((≤200 IU/ml)**	
<10	4 (50.0%)	4 (50.0%)	0.358
10 – 20	24(49.0%)	25 (51.0%)	
21 – 35	18 (40.0%0	27 (60.0%)	
> 35	2 (100.0%)	0.0%)(0	

Figure 4.1: Anti-streptolysin O "ASO" titer category distribution among studied sample according to age.



Description of Skin diseases and Renal diseases among study sample n=104 was distributed as follows: 19 People (18.3 %) of subjects had Skin diseases and 85 People (81.7%) of subjects had no Skin diseases. Whereas 3 People (2.9%) of subjects were Renal diseases and 101 People (97.1%) had no Renal diseases. **Table 4.6**

Table 4.6: Skin diseases and Renal diseases in the study sample.

Statements		**N**	**%**
Skin diseases	No	85	81.7%
	Yes	19	18.3 %
Renal diseases	No	101	97.1%
	Yes	3	2.9%

The relation between Anti-streptolysin O "ASO" titer category and Skin diseases in People without tonsillitis or pharyngitis group. There was a statistically significant relation between Anti-streptolysin O "ASO" titer category and Skin diseases in the People without tonsillitis or pharyngitis group ($P < 0.05$**). Table 4.7**

Table 4.7: The relation between Anti-streptolysin O "ASO" titer category and Skin Infections in People without tonsillitis or pharyngitis group.

Parameter		**Skin diseases (n=52)**		***P .value***
		Yes	No	
ASO **Titer**	**Positive(>200 IU/ml)**	11 (42.3%)	15(57.5%)	**0.012**
	Negative (≤200 IU/ml)	3(11.5%)	23 (88.5%)	

The relation between the Anti-streptolysin O "ASO" titer category and the use long of Benzathine Penicillin in people post tonsillectomy group. There was no statistically significant relation between Anti- streptolysin O "ASO" titer category and the use long of Benzathine Penicillin in the people post tonsillectomy group (P > 0.05). **Table 4.8**

Table 4.8: The relation between the Anti-streptolysin O "ASO" titer category and Use Long of Benzathine Penicillin in People Post Tonsillectomy group.

Parameter		**Use Long of Benzathine Penicillin (n=52)**		***P .value***
		Yes	No	
ASO Titer	**Positive(>200 IU/ml)**	2 (9.5%)	19(90.5%)	0.683
	Negative (≤200 IU/ml)	2(6.5%)	29 (93.5%)	

4.2. Discussion

The ASO titer can vary depending on the study population, geographic location, age group, and seasonal variation**(Saha et al., 2022).** The anti-streptolysin O test, an internationally standardized test is widely used for the detection of group A streptococcal infections and their sequelae. However, ASO titers can vary depending on the geographic location, age group of the study population, and climatic conditions. ASO titers of more than 200 Todd units are generally considered elevated in children and adults.**(Saha et al., 2022)**

ASO titer among people without tonsillitis and pharyngitis and people post tonsillectomy was as follows: 26 (50.0%) of People without tonsillitis or pharyngitis was ASO titer positive and 26 (50.0%) of those was ASO titer negative, while 22 (42.3%) of people post tonsillectomy was ASO titer positive and 30 (57.7%) of those was ASO titer negative.

This result reject research hypothesis that was ASO titer in people without throat infection and post tonsillectomy will be normal or negative, because found 26 (50.0%) of people without tonsillitis or pharyngitis were ASO titer positive and 22 (42.3%) of people post tonsillectomy were ASO titer positive

The results of this study showed that the Anti-streptolysin O test in People without tonsillitis or pharyngitis higher than values in People Post Tonsillectomy but not reaching statistical significance, (*P* value>0.05).

The study found that most of the study participants were aged 10 years to 20 years while those aged more than 35 years were the least., the majority of the participants were males. On the relationship between the demographic characteristics and Anti-streptolysin O "ASO" titer, the study found that aged 10 years to 20 the highest ASO positivity had no statistically significant relationship with Anti-streptolysin O "ASO" titer ($P > 0.05$)but, this finding agree with the finding of a retrospective study was done in the serology section of Department of Microbiology, Shaheed Hassan Khan Mewati Government Medical College, Nuh, Haryana**(Yadav et al., 2022),** that shows the highest ASO positivity was seen in 11–20 years age group. Also, this finding agrees with the finding of a retrospective study done on the blood samples received in the Department of Microbiology, PGIMS Rohtak, Haryana, India**(Rantz, Jacobs, & Kirby, 1943)**, that shows the highest ASO titer seen in 10-20 years of age group.

The current results showed the relation between Anti-streptolysin O "ASO" titer category and gender in the studied sample. There was a statistically significant relation between Anti-streptolysin O "ASO" titer category and gender in the studied sample ($P < 0.05$**).** This finding agrees with the finding of a retrospective cross-sectional study, which was performed in the central laboratory of Microbiology at Nepalgunj Medical College and Teaching Hospital, Banke, Nepal**(Khan, Singh, & Siddiqui, 2012)**, that shows the highest percentage of Anti-streptolysin O (ASO) antibody was found in female.

The current results showed the relation between Anti-streptolysin O "ASO" titer category and Skin diseases in People without tonsillitis or pharyngitis. There was a statistically significant relation between Anti-streptolysin O "ASO" titer category and Skin diseases in People without tonsillitis or pharyngitis ($P < 0.05$**).**

The current results showed the relation between the Anti-streptolysin O "ASO" titer category and the use long of Benzathine Penicillin in people post tonsillectomy. There was no statistically significant relation between Anti- streptolysin O "ASO" titer category and Use Long of Benzathine Penicillin in the People Post Tonsillectomy group ($P > 0.05$

Chapter Five

Conclusion & Recommendation

5. Conclusion & Recommendation

5.1. Conclusion

From the study findings, we concluded the following:

- From the results of this study, ASO antibodies were high in people without tonsillitis or pharyngitis and in people post tonsillectomy.
- The assessment of ASO antibodies in our study found Females showed more positivity compared to males, probably because mothers are in close contact with children and easily acquire the disease from them. ASO positivity was seen more in the 11–20 years of the age group which could be due to the development of a more competent immune system by this age.
- There is an association between the increase serum level of ASO with a skin infection in People without tonsillitis or pharyngitis.
- There is no effect of use long of Benzathine Penicillin on serum level of ASO.

It is important to note that commercially available kits react to all antibody classes IgG and IgM against streptococcal streptolysin. Hence, at any point, the titer reflects a previous infection but does not reflect if it is IgM recent infection or IgG old infection.

5.2. Recommendation

From the study findings, we recommend the following:

1. Depending mainly on high serum ASO titer for tonsillectomy is neither reliable nor acceptable, should perform throat swab culture and FNA culture along with ASO titer before doing tonsillectomy.
2. We recommend future studies to determine the reasons for that increase in ASO titer in females and, skin disease.
3. We recommend future studies to determine the relationship between ASO titer and use long of Benzathine Penicillin antibiotic.

Chapter Six

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