



Bacteriological and Immunological Study of Patients with Tonsillitis in Hila City

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Abstract: The current study was conducted to investigate the bacterial causes of tonsil infection which including chronic and acute tonsillitis. Two hundred twelve swabs were collected from the infected people with Tonsillitis in addition to 90 blood sample of people who reviewed Al-Hilla teaching hospital and Al-Noor hospital for children from October 2013 to April 2014 and 100 blood control. Culture investigations showed 197 positive bacterial growths. The bacterial growth include 147 Gram positive and 75 isolates Gram negative isolates, Gram-positive include *Streptococcus pyogenes* (20.2%), *Staphylococcus aureus* (19.3%), *Streptococcus pneumoniae* (15.3%) and *Streptococcus viridans* (11.2%). *Streptococcus pyogenes* show high percentage of isolate 20.2% while *S. viridians* show low percentage isolate (11.2%). While Gram negative include, *H. influenzae* (17.1%), *K. pneumoniae* (8.1%), *P. aeruginosae* (2.03), *E. coli* (3.6%). *H. influenzae* show high percentage of isolate (17.1%) while *E. coli* shows low percentage of isolates (3.6%). The concentration of cytokines was revealed in this study IL-17 showed increased at in concentration especially in age group 1-10 years and reached 55.25 pg/ml compared to control group. The Heat shock protein (HSP-27) showed increase in their concentrations in tonsillitis patient than in controls particularly at age group 21-30 years which reached 22.25 pg/ml.

Keywords: Tonsillitis, IL-17, HSP27.

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Introduction:

Tonsillitis is a common respiratory tract disease that affects both children and adults most cases of tonsillitis occur due to infection by bacteria, viruses, allergies and respiratory problems (1). Tonsillitis is known to interact with the lymphatic tissue in the tonsils with the factors causing inflammation such as bacteria and as a result of this interaction symptoms are characterized by high temperature and enlargement of the tonsils and congestion and weakness in the performance and guest member and the two types of inflammation of the tonsils:

1. Acute Tonsillitis this type suddenly on the tissue tonsils and the symptoms of inflammation of the

general symptoms may disappear after treatment (2).

2. Chronic tonsillitis it is continuous and repeated, with short periods of time and is more dangerous to the tissue of the first type .The most important pathogens for tonsillitis are *Beta-hemolytic streptococcus*, *S. aureus*, *H. influenza* and *Pneumococci*(3). The tonsils form the first line of defense of the body's immune system but also a place with repeated inveterate inflammatory operation and controversies in treatment. It act the effectors of systemic humoral and cellular immunity (4). Interleukin-17 is primary for host protection opposed to numerous microbes, especially extracellular bacteria and fungi.

Interleukin-17 Interleukin has great importance in protecting host from infection (5). Heat shock proteins (HSP-27) are very preserved proteins set up in prokaryotes and eukaryotes (6). The aim of this study is to diagnosis of bacterial species that causes Tonsillitis, and detection of some immunological aspects in Tonsillitis patients.

Materials and methods:

The study inclusive 212 swabs from patient's tonsillitis who reviewers to Hilla General teaching hospital and Al-Noor hospital for children for age of patients from (1-60) years. Swabs were collected from patient tonsils by disposable transport media sterile. Swabs were taken and close it until transported to immunology laboratory in college and culturing on different media for 24hours at cultivate 37 °C for bacterial diagnosis. Microbiological analysis was done and the organisms were identified by direct Gram staining, culture methods on Nutrient Agar, Blood Agar, MaCConkey agar, Chocolate Agar Medium, Simmon Citrate Medium and Eosin Methylene Blue (EMB) Agar at 37 for 42h. And different biochemical tests like catalase test, oxidase, indole, Simmon Citrate, coagulase, voges-proskauer (VP),

methyl red (MR),capsule test, motility test, Bacitracin sensivity test, Urease and were performed for the identification of the various bacterial pathogens after their isolation.as well as 90 blood samples were collected from patients aged 1-60 years of 120 males and 90 females.

Blood Samples:

Five ml of blood were collected from 90 blood samples in patients with tonsillitis by disposable syringe, blood was put in tube without anticoagulant. The serum was separated by centrifugation at 3000 rpm for 5mint within 2-3 hours after collection. Serum levels of IL17 and hsp27were measured by enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative saandwich immunoassay using Peprotech (USA) kit.

Results:

Distribution of Tonsillitis According to Age:

Results of present study showed that occurrence of tonsillitis among the patients according to age group 1-10 years (33 %), 11-20 years (25.9 %) and the least incidence years in 51-60 years, (4.7%) as shown in (Figure 1).

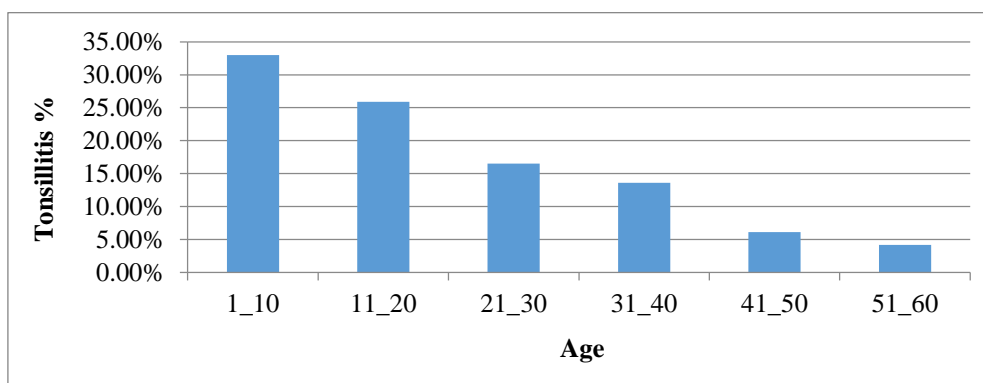


Figure (1): Distribution of tonsillitis patients according to age.

Distribution of Tonsillitis According to Gender:

This study was showed the prevalence of tonsillitis among the male

was 120 (56.6%) compared to in females 92 (43.3%) as it is clarified in (Figure 2).

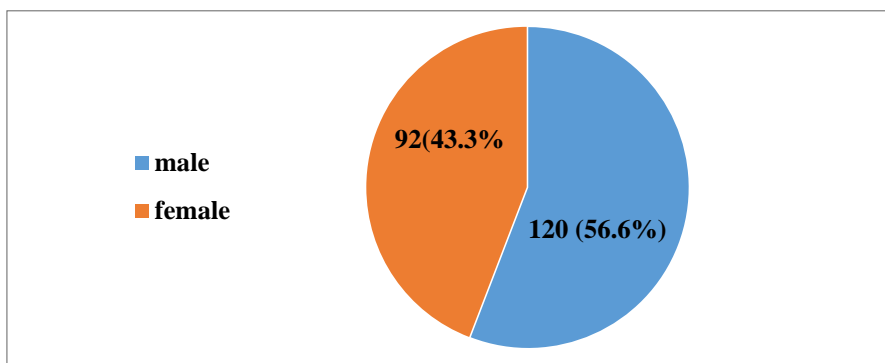


Figure (2): Distribution of tonsillitis according to Gender.

Bacteriological Identification:

Isolation of Bacteria:

In the present study, Culture of samples showed 197 positive results and 15 with no growth, as shown in

(Figure 3). The positive culture show distribution of 147 gram positive bacteria and 75 gram negative depending on gram stain investigation, as in (Figure 4).

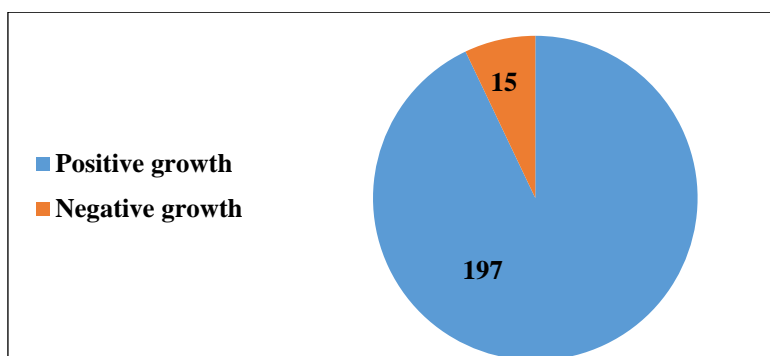


Figure (3): Distribution of bacterial growth among cultured samples.

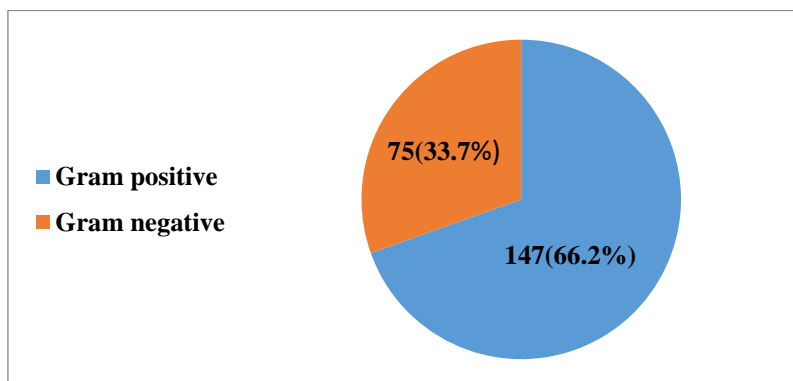


Figure (4): Number and percentage of bacterial isolates according to gram stain investigation.

Isolation of bacterial causative in Tonsillitis:

The bacteriological study included acute and chronic Tonsillitis. In acute tonsillitis results indicated the occurrence of *Staphylococcus aureus* 22(23.1%), followed by *Streptococcus pyogenes* 20(21%), *Streptococcus pneumoniae* and *Haemophilus influenzae* 16(16.8%) for each of them, *Streptococcus viridians* 9 (9.4%), *Klebsiella pneumoniae* 5(5.2%),

Pseudomonas aeruginosa 4(4.2%) and *Escherichia coli* 3(3.1%). Whereas in chronic tonsillitis indicated the occurrence of predominant *Streptococcus pyogenes* 25 (19.6%), followed by *Staphylococcus aureus* 21(16.5%), *Haemophilus influenzae* 22 (17.3%), *Streptococcus pneumoniae* 18(14.1%) *Streptococcus viridians* 16 (13.5%), *Klebsiella pneumoniae* 13(10.2%) *Pseudomonas aeruginosa* 7(5.5%) and *Escherichia coli* 5(3.9%) (Table 1).

Table (1): The prevalence and percentage of bacteria isolated from acute and chronic tonsillitis patients.

Type of bacteria	Acute	Chronic	Total
<i>S. pyogenes</i>	20(21%)	25(19.6%)	45(20.2%)
<i>S. aureus</i>	22 (23.1%)	21(16.5%)	43(19.3%)
<i>H. influenzae</i>	16(16.8%)	22(17.3%)	38(17.1%)
<i>S. pneumoniae</i>	16(16.8%)	18 (14.1%)	34(15.3%)
<i>S. viridans</i>	9(9.4%)	16 (13.5%)	25(11.2%)
<i>K. pneumoniae</i>	5(5.2%)	13(10.2%)	18(8.1%)
<i>PS.aeruginosa</i>	4(4.2%)	7(5.5%)	11(4.9%)
<i>E. coli</i>	3 (3.1%)	5(3.9%)	8(3.6%)
Total number	95	127	222

Immunological Assay for Tonsillitis patients:

Role of IL-17 in Tonsillitis patients:

The study showed high values of IL-17in patient's serum compared to healthy and high values in age class (1-10 years) reach55.25pg/ml compared

to in healthy and in same classes 19.5 pg/ml. With a significant increase in mean values ($P < 0.05$), between values, IL-17in patients compared to healthy and also between the age group (51-60 years) shows low values of IL-17which reach 13.25pg/ml compared to in healthy, as shown in (Table 2).

Table (2): IL-17values in Tonsillitis patients and control.

Age group	IL-17 pg/ml	IL-17 pg/ml
	Patient (M± S.D)	Control (M±S.D)
1-10	55.25± 27.87	19.5±9.88
11-20	23.75±7.5	15.75±4.99
21-30	38.77±25.15	27±5.71
31-40	26.75±7.1	18.75±5.37
41-50	14.25±10.71	13±5.71
51-60	13.25±5.37	12±8.90

LSD= 7.4, ($P < 0.05$).

Role of HSp-27 in Tonsillitis patients:

Results showed high values of HSP-27 in patient's serum compared to healthy and high values in age group (21-30 years) which reach 22.25pg/ml compared to in healthy and in same

group(16.95 pg/ml). Show significant increase in mean values ($P < 0.05$), between values HSP-27 in patients compared to healthy and also between age group show (1-10 years) low values of HSP-27 at reach 17pg/ml compared to healthy, as show in (Table 3).

Table (3): HSP-27 values in Tonsillitis patients and control.

Age group	HSP-27 pg/ml	
	Patient (m± S.D)	Control (M±S.D)
1-10	17±2.94	12±7.16
11-20	21±7.16	15.8±2.69
21-30	22.25 ±6.84	16.95±5.17
31-40	19.12±7.06	16.19±5.125
41-50	18.5±6.52	15.02±5.12
51-60	17.5±6.33	14.60±5.03

LSD=6.1, ($P < 0.05$).

Discussion:**Patients Demography:****Distribution Tonsillitis According to Age:**

Results showed that the incidence of tonsillitis in relation to population distribution was found to be different. Among reported age groups, maximum tonsillitis cases were noticed in the class age group 1-10 years (33%), 11-20 years (25.9 %) and the minimum rate years 51-60 years (4.7%). The prevalence were revealed patients in class (1-10 years) because it can be associated with the increased activity of children at this age, which gives a greater chance of exposure to infection than other ages. In addition, this is the school age in which children mix and communicate with one another in the classroom without ignoring the role of transportation within the family (7). Also (8) found that among tonsillitis patients in children is between age (1-10 and 11-20). While (9) found that the tonsillitis percentage was 62% in age

group 1-10 years and 38% in age group 11-20 years (10,11) founds the age has been reported to be an important factor in the microbial etiology of GA β HS pharyngitis as well as in its carriage state. Among children was higher in the age group of 6-10 (40.4%) compared to 23.8% and 20.1% in the age groups 1-5 years and 11-15 years ,respectively.

Distribution Tonsillitis According to Gender:

The study show that Tonsillitis was occurrence in male at 120(56.6%) compared to female 92 (43.3%). The result showed the occurrence of tonsillitis in males compared to females because it appears that the physiology of masculinity and hyperactivity in comparison with females' inclusive smoking and age has a role in the occurrence of tonsillitis, particularly among older patients. Smoking has a negative effect on the health of the gums, as it affects the delicate plants in the oral cavity (12). This results were agreed with (13) who found 63% male and 37% female. (14). Found Sex wise

distribution showed that 245(29.1%) male were females 221(28.1%) (15) showed the distribution of tonsillitis was more in male patients (55%) compared to female patients (45%) probably because number of patients admitted were more than female patients.

Bacteriological Identification:

Isolation of Bacteria:

In this study, the results of bacteriological culture show 197 positive growth and 15 no growth. The cause for not growing is probably because patients even take antibiotics before diagnosis or cause tonsillitis of the virus (16)

The present study show 147 (66.2%) gram positive bacterial isolates and only 75(33.7%) belonged to gram negative bacterial isolates. As the gram positive bacteria are the normal colonies of skin and other oral cavities, their number is probably found more than gram negative bacteria (17).

Isolation of bacterial causative in Tonsillitis:

Results showed that *Streptococcus pyogenes* was the predominant isolates in tonsillitis 45 (20.2%) followed by *Staphylococcus aureus* 43(19.3%), *Haemophilus influenzae* 38(17.1%), *Streptococcus pneumoniae* 34(15.3%), *Streptococcus viridans* 25(11.2%), *Klebsiella pneumoniae* 18(8.1%), *Pseudomonas aeruginosa* 11(4.9%) and *Escherichia coli* 8 (3.6%). Several studies refer to similarity in bacterial isolates with this study (18,19) which were found *Streptococcus pyogenes*, formed (36%), (25%) respectively. The

prevalence of GAS in this study is higher may be because it depends on the method of investigation. For instance, the use of sheep blood instead of human blood will increase the rate of GAS isolation (20). Where many other studies pointed variation in bacterial isolation ratio with the present study (21,22) who found that the rate of GAS in tonsillitis is (9.2%) and (10.9%) among school children respectively. Moreover, GAS isolation at lower in studies this may be attributed in that some studies are focused on children only. Also the recurrent usage of antibiotics without consulting the physician plays a major role in the reduction of the opportunity of these bacteria (23). In this study represented *Staphylococcus aureus* is the second causes of tonsillitis and isolates 43(19.3%). Several studies refer to similarity in bacterial isolates ratio with this study (24,25) found *Staphylococcus aureus* ratio (21%), (26 %) ,respectively .*Staphylococcus aureus* isolates could be due to the fact that *S. aureus* is part of the oropharynx normal flora. On the other hand it is emphasized that among any large population of *S. aureus* isolates, resistance to fusidic acid will occur (26). The virulence factor include both structural components of bacterial cell wall, such as peptidoglycan and lipoteichoic acid, and surface proteins including protein A, fibronectin-binding protein and clumping factor, while secreted proteins include invasins (that promote bacterial spread in tissue such as leukocidin, staphylokinase, hyaluronidase), membrane-damaging toxins (that lyses eukaryotic cell membranes such as hemolysins, leukotoxin and leukocidin) and tissue-damaging toxins (such as enterotoxin A-G, toxic shock syndrome-toxin

(TSST-1), and V8 protease(27). In this study, *S. pneumoniae* isolates 34(15.3%) and *S. viridans* 25(11.2%) these results when compared to other studies are correlated with (28).

The most predominant isolates of gram negative bacteria found that *H. influenzae* the most bacterial isolates from chronic and acute tonsillitis 38(17.1%). These results were agreed with (29) who found *H. influenzae* to be the most gram negative bacteria isolates (32%). While (30) who reported that *H. influenzae* was the most common isolates from Tonsillitis. *Haemophilus . influenzae* commensal that inhabits the nasopharynx and upper airways of children and healthy adults and infections frequently persist for long periods of time and can be highly resistant to immune clearance and antibiotic treatment at least in part due to persistence of bacteria within biofilm communities(31). The study shows *Pseudomonas aeruginosa* isolates 11(4.9%) and *Klebsiella pneumoniae* 18(8.1 %) These results were agreed with (32) who showed two gram negative bacteria ,*Klebsiella pneumoniae*, (8.82%),and *Pseudomonas aeruginosa* (2.94%) isolates in tonsillitis (33) who found *Pseudomonas aeruginosa* isolate in tonsil 3 (7.69%) and *Klebsiella pneumoniae* 7 (17.95%) which are in agreement with this study. Most *P. aeruginosa* strains involved in infections are both invasive and toxigenic, as a result of the production of surface virulence factors (allowing bacterial attachment, colonization and invasion) and secreted virulence factors which damage tissues or trigger the production of cytokines (34). In the present study, *E.coli* isolates are 8(3.6%). This is was in agreement (35) who found *E .coli* isolates (2.86%).

Immunological Assay for Tonsillitis patients:

Role of IL-17 in Tonsillitis patients:

The study showed high values IL-17 in patient's serum compared to healthy. Recorded high values in classes age (1-10 years) reach at 55.25pg/ml. IL-17 have important function in defense against streptococcus pyogens .Interleukin -17 works via rising output chemokine in late-kind response in different tissue to conscript neutrophil and mononuclear cells to the site of inflammation identical to TNF (36). IL-17 serves as a proinflammatory cytokine in response to the attack of extracellular pathogens and stimulates the destruction of pathogens (37). IL-17 works jointly with TNF α and induce the output of other cytokines as IL6, G-CSF, GM-CSF, IL1 β , TGF β , TNF α , chemokines including IL8, GRO- α , and MCP-1, and also prostaglandins as PGE2 (38).

IL-17 especially in mucous sites such as the lung, gastrointestinal tract and oral cavity. The cells output IL-17 are supplement in the mucosal surfaces(39).

Role of Hsp-27 in Tonsillitis patients:

In this study showed high values in Hsp-27 in patients compared to healthy. Recorded high values in class's age (21-30 years) reach at 22.25pg/ml. Hsp-27 play important role in modify operation of immunological through expressible in the standard of antigen presentation and in transfer of peptides to the major histocompatibility complexes (MHC) (40) HSP-27 may be constitutively expressed at low levels in normal cells and increase in response to various

environmental stresses such as heat shock inflammation, infections by viruses or bacteria, irradiation, anoxia, and exposure to metals (41). Recent studies suggest that interleukin, in addition to the importance of prevention during exposure to heat, shock and various stress, whether environmental or physiological stress, shows many range of functions inclusive regulation of cell growth and differentiation and of cytoskeleton dynamics signal transduction and defense versus apoptosis induced by various factors(42).

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