The Impact of IL-6 serum level on Tonsillitis and Tonsillectomy Patients infected with Streptococcus pyogenes

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Abstract. Acute tonsillitis is a primary medical care consultation that may develop to recurrent tonsillitis (RAT), chronic tonsillitis and ~30% of patients require a tonsillectomy, which is associated with a high frequency of missed work or influence on quality of life. The present study included 100 patients attending to Al-Saddar Medical city in ENT unit in Najaf governorate. The Patients were classified into two groups: seventy patients with tonsillitis who were subdivided into (36) as acute tonsillitis, (23) patients with chronic tonsillitis and (11) with recurrent tonsillitis as well as thirty patients with tonsillectomy. Swabs were taken from tonsil surface and from tonsil core then culturing on different media for 24 hours at 37 °C for bacterial growth and diagnosis by morphological, biochemical and Vitek 2 system, two ml of a venous blood sample was collected from each patient and control for ELISA testing to determine the level of IL-6. This study has shown that higher frequency of tonsillitis in males. The highest percentage of patients appeared with Grade 3 (33.6%), followed by Grade 2 (22.7%), in tonsillitis patients and grade 4, grade 3 in tonsillectomy patients. Also the age group of 1-10 years more frequency 34.4%. Culture investigation shows 16 (16%) swabs gave negative bacterial growth and 84 (84%) swabs gave positive bacterial growth from which about 108 isolates as poly and monobacterial growth (data not show). The 108 isolates appear that G+ ve bacteria recorded 88 (81.5%) with more frequency to S.pyogenes 35 (32.4%) isolates. The level of IL-6 highly increase in serum of tonsillitis and tonsillectomy patients compared with healthy controls. IL-6 serum level was highly increased among the patients in grade 3, 4 and highly significant increase in recurrent and chronic patients. As a result S. pyogenes is the most predominant bacterial agents in surface and core tonsillitis. IL-6 play important role in healing inflammation of tonsils.

Keywords. Tonsillitis, S. pyogenes, tonsillectomy, recurrent, IL-6.

1. Introduction
Tonsillitis is a common respiratory tract disease that affects both children and adults due to infection by bacteria, viruses, allergies that interact with the lymphatic tissue in the tonsils causing inflammation, it is a public health problem because of its frequency, socio-occupational and economic impact [1]. About 9 million new cases of tonsillitis are diagnosed each year [2]. The most important pathogens for tonsillitis are Group A beta hemolytic streptococcus (GABHS), or Streptococcus pyogenes, a human pathogen responsible for a broad range of infections and is an important cause of global morbidity and mortality [3].
S. pyogenes is a strict human pathogen responsible for >700 million infections and ~517,000 deaths annually worldwide [4]. Human infections range in severity from relatively mild conditions such as pharyngitis to life-threatening septicemia and necrotizing fasciitis/myositis. GAS also causes skin infections such as impetigo and erysipelas and post-infection sequelae, including rheumatic fever, rheumatic heart disease, and glomerulonephritis [5].

GAS can survive in lymphoid tissue and induces a humoral immune response by induces a CD4+ T cell response, which express T cell antigen receptors (TCR) that recognize short peptides bound to MHC-II molecules (pMHCII) expressed by host antigen-presenting cells that stimulate T- cells to proliferate and differentiate into Th1, Th2, or Th17 effector cells that produce cytokines such as IL-6, IL-10 or IL-17 respectively, which help to eliminate the pathogen [6]. Therefore, this study aimed to study prevalence of S. pyogenes that causes tonsillitis and determine the correlation between type of disease acute, chronic, and recurrent as well as tonsillectomy with IL-6 serum level.

2. Materials and Methods

2.1. Study Subjects and Sample Collection
A total of 100 subjects were included in this study; Patients admitted to Al-Sadder medical city, ENT unit, in Al–Najaf province during the period from September 2019 to March 2020. The clinical diagnosis done by ENT specialist. Swabs were taken from tonsil surface without touch any adjacent surfaces and from tonsil core immediately after tonsillectomy, in these samples, the excised tonsils were sterilized using iodine solution and opened with a sterile scalpel and swab taken from the fibrosis present in the tissue using disposable sterile transport medium swab, then culturing on different media for 24 hours at 37 °C for bacterial growth and diagnosis by Gram stain, cultural morphological, biochemical and Vitek 2 system. Two ml of a venous blood sample was collected from each patient and control groups was transferred into gel tube, left 10 minutes for clotting then centrifuge at 3000 rpm for 10 min, and the serum was collected in sterile Eppendorf tubes and kept at -20 C̊ for ELISA to determine the level of IL-6 according to (Elabscience, USA).

2.1.1. Ethical approval
This study was ethically approved by the medical ethics committee in Al-Sadder medical city, Najaf. Moreover, adult patients and the parent of the children patients gave the informed consent before they gave the samples.

2.1.2. Statistics analysis
The results are presented as means and statistical with standard error (S.E.) and were analyzed using one-way analysis of variance (ANOVA) test via Graphpad prism 5.04. p < 0.05 was considered significant.

3. Results

3.1. Distribution of Study Subjects According to Type of Disease and Gender
A total of 100 patients with tonsils infection that divided into two main group, 70 (70%) tonsillitis patient that further divided into acute tonsillitis with 36 (51.4%), chronic tonsillitis were 23 (32.8%) and recurrent tonsillitis were 11 (15.8%) and 30 (30%) tonsillectomy patients, In addition to 30 apparently healthy subjects as controls group as show in Table (1). The gender distribution revealed that 70 tonsillitis patients from which 33 (47.1%) were females and 37 (52.9%) were male. In tonsillectomy cases 14 (46.7%) were females and 16 (53.3%) were males. The controls recorded 15 (50%) for both males and females as show in table (1).
Table 1. Distribution the study subjects according to type of disease and gender

| Sex     | Tonsillitis |          | Tonsillectomy |          | Control |
|---------|-------------|----------|---------------|----------|---------|
|         | Acute No (%)| Chronic No (%)| Recurrent No (%)| Tonsillectomy No (%)|          |
| Female  | 16 (44.4%)  | 11 (47.8%) | 6 (54.5%)     | 14 (46.7%) | 15      |
| Male    | 20 (55.6%)  | 12 (52.2%) | 5 (55.5%)     | 16 (53.3%) | 15      |
| Total   | 36          | 23       | 11            | 30       | 30      |

3.2. Distribution of Patients According to Grade of Disease
Patients with Tonsillitis are divided into several grade with different numbers and percentages, the highest percentage of patients appeared with Grade 3 (42.8%), followed by Grade 2 (31.4%), Grade 1 (20%), and Grade 4 (5.8%), While in tonsillectomy the grade 4 (43.4%) and 3 (33.3) was high frequency then Grade 2(23.3%) as shown in figure (1).

Figure 1. Distribution of patients according to grade of disease

3.3. The Diagnostic Characteristics of S. pyogenes
The present study illustrated that S. pyogenes recorded 35 isolates distributed as following: 11 (31.4%) isolated from core after tonsillectomy and 24 (68.6%) from surface of tonsils: 10 (28.3%) from acute tonsillitis, 5 (14.7%) from recurrent and 9 (25.6%) from chronic tonsillitis as show in figure (2).

Figure 2. The frequency of S.pyogenes according to type of infection

3.4. Estimation the Level of IL-6 in Patients and Controls
The result indicated that a significant difference in the serum level of IL-6 between patients and healthy controls. It is clear that IL-6 has been increased in the serum of Tonsillitis patients to 86.31 pg/ml, tonsillectomy patients 93.65 pg/ml in compared with 23.94 pg/ml in healthy control as shown in Figure (3).

![Figure 3. The serum IL-6 levels in tonsillitis, tonsillectomy patients and healthy controls](image)

The result indicated increased serum level of IL-6 in acute tonsillitis patients was (25.34 ± 2.89) and highly increased in serum level in chronic, and recurrent tonsillitis patients, which about (31.74 ± 4.09) pg/ml, (29.54 ± 3.78) pg/ml respectively as shown in figure (4).

![Figure 4. IL-6 levels in acute, chronic and recurrent](image)

3.5. Evaluation the IL-6 Level in Patients According to Grade of Disease
The present study show that IL-6 serum level was highly increased among patients in grade 3, 4 (45.65 ± 5.61, 70 ± 4.32 pg/ml) while less increased in patients within grade 1, 2 (30.22 ± 3.80, 34.33 ± 4.5 pg/ml) P ≤ 0.05 as show in figure (5).

![Figure 5. The percentage of serum IL-6 in patients according to grades of disease](image)
3.6. Correlation between IL-6 serum level in patients infected with S. pyogenes and patients infected with other pathogens

The result appeared that IL-6 highly increased in patients infected with S.pyogenes (99.82 ± 6.47) pg/ml in compare with patients infected with other pathogens which about (80.38 ± 5.76) pg/ml.

![Image](IL-6 level)

**Figure 6.** IL-6 serum level in patients infected with S.pyogenes and with other pathogens

4. DISCUSSION

Tonsillitis has significant impact on health status and quality of life as it causes significant morbidity and time lost for school or work.

4.1. Distribution of study subjects according to type of disease and gender

This study showed high incidence in acute tonsillitis than other type of tonsillitis and in men than female. Abraham et al. (2019) illustrated male were more frequently infected with tonsillitis with percent 51% and the female were 49 %. Abouzied and Massoud, (2008) they state that tonsillitis more common in males since they may have a weaker immune system and since that manifestation of a hyper-immune state augmented by the effects of estrogen in females. This result is line with previous study by [7] who observed the acute supportive tonsillitis record 41% then chronic tonsillitis and in line with [8]; they confirmed that male-recorded 53.7% of tonsillitis patients and 46.3% female. And disagreement with [7] observed that females were more predominantly, infected with tonsillitis than males.

4.2. Distribution of patients according to grade of disease

The grade of the palatine tonsils is usually defined by the volume of space that is claimed by the tonsils in the pharyngeal airway and several most patients present with either an excess amount of soft tissue or an under developed bony skeleton that leads to the narrowing of the pharynx [9]. This results similar with [10] they founded that male more than females patients and the grade 2 and 3 the highest incidence then grade 1 and 4 with frequency 35.3%, 34.1%, 28.2%, and 2.4% respectively and illustrated that patients with greater tonsil grade were likely to benefit from tonsillectomy with additional changes in the pharyngeal cavity. Therefore, tonsil grade obtained via physical examination was conducive to select surgical candidates with a high success rate of treatment. Ng et al. [11] they confirmed that patients with tonsil grades 3 and 4 had a significantly greater volume than those with grades 1 and 2, which revealed that patients with greater tonsil grade were likely to benefit from tonsillectomy with additional changes in the pharyngeal cavity.

4.3. Bacterial distribution from tonsillitis and tonsillectomy specimens

In this study, the 108 isolates appear that G+ ve bacteria recorded 88 (81.5%) with more frequency to S.pyogenes 35 (32.4%). In local study done by Najim et al. [12] in Basrah, they found that males were more frequency in tonsillitis and tonsillectomy with 51% and confirmed that gram's positive bacteria were the commonest microorganisms, was S. pyogenes with followed by S.aureus was isolated in
chronic tonsillitis patients also, tonsillectomy subjects recorded the predominant Streptococcus (α-haemolytic) isolates followed by S. epidermidis. Abraham et al. [13] they appeared that tonsillitis infection were common prevalent in young individual with male preponderance was found (51%), the most affected age group was 1-10 years (42.6%), also identify the causative agents which were Coagulase negative staphylococcus 41.7%, S. pyogenes 40. In other study, in Baghdad done by [7] they explain that S.pyogenes was the predominant causative agents in acute supplicative tonsillitis patients with frequency (73.9%), S.pneumonia recorded (3.1%), S.aureus recorded (20%)

4.4. Estimation the level of IL-6 in patients and controls

In this study, it is clear that IL-6 has been increased in the serum of Tonsillitis patients, tonsillectomy patients in compared with in healthy control. This result was line with other study by Skovgaard et al. [14] observed IL-6 were significantly induced after infection in most tissues of tonsils. The high concentrations of IL-1, IL-6 and TNF-α in adenotonsillar tissues, believing it to be an expression of local overproduction due to monocyte-macrophage activation resulting from repeated stimuli from pathogen agents [15].

In this study increased serum level of IL-6 in acute tonsillitis patients was concordance with other study by Skovbjerg et al. [16] they showed tonsillar secretions from patients with acute tonsillitis regardless of age had significantly higher levels of IL-1β, IL-6, IL-8, IFN-γ, and PGE2. And in line with Smiyan et al. [17] revealed that the level of IL-6 in patients with chronic tonsillitis increased significantly in the first days of hospitalization and decreased after the standard treatment, but did not reach the levels of healthy. Also, [18] confirmed that levels of IL-1, IL-2, and IL-6 showed a significant increase in the recurrent tonsillitis group when compared to the hypertrophy group. In addition, Murat-Unal et al. (2002) they evaluate serum level of interleukins IL-1β, IL-4, IL-6, and IL-8 in chronic tonsillitis patients before and after tonsillectomy and found that IL-1β and IL-6 levels were significantly higher than the control levels (p < 0.05) in preoperative serum samples.

4.5. Evaluation the IL-6 level in patients according to grade of disease

The present study show that IL-6 serum level was highly increased among the patients in grade 3, 4 while less increased in patients within grade 1, 2. This results similar to study done by [19] who found that grade 4 was high frequency than other in tonsillectomy patients as well as the serum level of IL-6 was higher in grade 4 than other grade. Also, Deborah et al., [20] they confirmed that IL-6 levels were higher in severe as compared to mild infection. The higher IL-6 levels with grade 3 and 4 tonsils may be due to increased tissue bulk as hyperplastic tissue that maintains most of immunologic function [21].

4.6. Correlation between IL-6 serum level in patients infected with S.pyogenes and patients infected with other pathogens

The most common pathogen in bacterial tonsillitis is (GABHS), which appear high TNF-α, IL-6, serum level in tonsillitis patients infected with GAS than non-A (groups B, C, F and G) hemolytic streptococcus [22]. GAS infection of HEp-2 epithelial cells induced a significant proinflammatory response and secretion of IL-6 and TNF-α [23]. Blood samples from patients with acute GAS tonsilitis showed high levels of the proinflammatory cytokines TNF-α and IL-6 (Bell et al., 2012).

Rasheed et al. [24] they showed that systemic and mucosal IL-6, IL-10, IFN-γ were found in higher concentration in RT-patients harbors S.pyogenes in compare to those harbors S. aureus in their tonsils. Tonsils infected by S.pyogenes due to increasing expression of pro-inflammatory cytokines [25]. In response to the attack of extracellular pathogens and stimulates the destruction of pathogens there are a interaction between cytokine such as IL-17 works jointly with TNF-α and induce the output of other cytokines as IL-6, IL-1β, TNF-α and chemokines including IL-8, and MCP-1 [26].

Polymicrobial infection with an insufficient host defense is the most frequent reason for tonsillar inflammation that induced by a release of various cytokines and chemokines, such as TNF-α, IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12, and so on [27].
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