

## Research Paper



## Evaluation of some immunological parameters against streptococcus pyogenes in tonsillitis

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### ABSTRACT

This study was designed to evaluate some immunological parameters in patients with chronic tonsillitis. Swabs were obtained from the tonsils of 120 patients with chronic tonsillitis to detect streptococcus pyogenes and blood samples collected to detect the immune parameters in the patient's serum. The results of culture showed that 60 samples (50%) were positive to streptococcus pyogenes. Significant differences were observed in ASO titer levels between the tonsillitis group (400 IU, interquartile range: 400-1600) and the control group (200 IU, interquartile range: 0), with a p-value <0.05. However, no statistically significant differences were observed in TNF- $\alpha$  levels between the two groups, with a median of 248 pg/ml (interquartile range: 125) in the tonsillitis group and 209 pg/ml (interquartile range: 112) in the control group (p>0.05). Nevertheless, a significant decrease in IL-10 levels was observed in the tonsillitis group (51.8 pg/ml, interquartile range: 29.4) compared to the control group (81.4 pg/ml, interquartile range: 36.4), with a p-value <0.05.

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## 1. INTRODUCTION

The tonsils are a group of lymph nodes situated at the back of the throat, in a delicate area that is part of the upper respiratory system [1]. In the pharynx, the tonsils create a ring called the Waldeyer's ring. This ring serves as the body's first line of defense against a wide range of infectious agents, including viruses

and bacteria. It also plays a role in the production of antibodies, including IgA [2]. And this can be because of the lymphocytes being stimulated by the same antigens over and over again [3]. Staphylococcus aureus and Group a beta-hemolytic streptococci (GABHS) are the major culprits when it comes to acute and chronic tonsil inflammation [4], [5].

A delicate mechanical balance regulates the infection-specific immune responses by promoting the synthesis of the and the cytokines. A cell-mediated response is used to initiate an immune response by the cytokines generated from the (TNF- $\alpha$ ). According to [6], the cells trigger a humoral response by secreting IL-10. It is possible to utilize clinical criteria, such as white blood cell total and differential counts, to diagnose bacterial infections [7]. Write Acute and chronic tonsillitis patients' immunological parameter levels were the focus of the current research, which also sought to isolate and diagnose streptococcus pyogenes in tonsillitis patients. One kind of bacteria, S. pyogenes, was gram-positive. According to [8], [9] streptococcus is a dangerous human pathogen that may infect the tonsils and skin, causing a wide range of illnesses, from non-communicable sore throat and fever to potentially fatal and infectious invasive infections. It was one among the top 10 infectious diseases that caused human mortality [10]. Eleven of these virulence agents have been designated as super antigens, as is typical of most pathogens [8].

## 2. RELATED WORK

A study [11] found that tonsillitis patients had increased levels of TNF- $\alpha$ , indicating that the tumor necrosis agent stimulates blood vessel linings and increases permeability, leading to enhanced immunity. Additional studies have demonstrated that IL-10 has a variety of anti-inflammatory properties and plays an important function in infection as a regulator of primitive immunity. IL-10, which is produced in reaction to bacteria and their products, plays an important part in the pathogenic process [12].

## 3. METHODOLOGY

A total of 120 samples of chronic tonsillitis patients were collected at the Tikrit Teaching Hospital in Tikrit city. For the period from the beginning of October (1/10/2023) to the end of December (24/12/2023) and for different age groups (5-15 years) for both genders. The samples were taken with a swab from the tonsils. Were cultured on blood agar and incubated at 37°C for 24-48 hours. The growth diagnosed using biochemical tests and Vitek 2. 5ml venous blood was collected from all patients and healthy people as control group, and put in a jel tube and Centrifuged. The serum was immediately stored in an Eppendorf tube and frozen at -20C° until assayed for immunological parameters. ASO titer was measured by serial dilution assay test. The level of interleukin-10 and tumor necrosis factor-alpha was assayed by Elisa kits accordance with the instructions of the Chinese company sun long biotech.

## 4. RESULTS AND DISCUSSION

### Results

This study included 60 patients with tonsillitis and 30 individuals as a control group for comparison. Regarding age, there was no significant difference between the patient group (9 years, interquartile range: 6.25 years) and the control group (8.5 years, interquartile range: 7.5 years), with a p-value greater than 0.05. The gender distribution (male/female) was also similar between the two groups, with a male-to-female ratio of 25/35 in the patient group and 15/15 in the control group (p>0.05, Table 1).

Table 1. Demographic Characteristics of Study Participants

Parameter	Control (n= 30)	Tonsillitis (n= 60)	P-value
Age (years)	8.5 (7.5)	9 (6.25)	0.611
Sex (M/F)	15/15	25/35	0.453

Table 2 shows the difference in concentrations of immunological markers between patients and control group. Significant differences were observed in ASO titer levels between the tonsillitis group (400 IU, interquartile range: 400-1600) and the control group (200 IU, interquartile range: 0), with a  $p$ -value  $<0.05$ . However, no statistically significant differences were observed in TNF- $\alpha$  levels between the two groups, with a median of 248 pg/ml (interquartile range: 125) in the tonsillitis group and 209 pg/ml (interquartile range: 112) in the control group ( $p>0.05$ ). Nevertheless, a significant decrease in IL-10 levels was observed in the tonsillitis group (51.8 pg/ml, interquartile range: 29.4) compared to the control group (81.4 pg/ml, interquartile range: 36.4), with a  $p$ -value  $<0.05$ .

Table 2. Levels of Immunological Biomarkers in Serum Samples of the Participants

Parameter	Control (n= 30)	Tonsillitis (n= 60)	P-value
ASO titer (IU)	200 (200, 200)	400 (400, 1600)	$<0.001$
TNF- $\alpha$ (pg/mL)	209 (179, 291)	248 (197, 321)	0.342
IL-10 (pg/mL)	81.4 (61.8, 98.1)	51.8 (40.2, 69.3)	$<0.001$

Data were presented as median (IQR).

In Table 3 and Figure 1, the concentrations of immunological biomarkers are presented by gender for both patients and healthy controls to assess the impact of gender on the described covariance. The results showed no statistically significant differences for all variables ( $p>0.05$ ).

Table 3. Serum Levels of Immunological Biomarkers in of the Participants by Sex

Parameter	Control (n= 30)		P	Tonsillitis (n= 60)		P
	Male	Female		Male	Female	
ASO titer (IU)	200 (0)	200 (0)	0.999	1200 (800)	800 (400)	0.454
TNF- $\alpha$ (pg/mL)	210 (127)	190 (101)	0.321	248 (107)	247 (139)	0.652
IL-10 (pg/mL)	89.5 (33.6)	69.7 (24.6)	0.143	46.2 (31.7)	53.3 (25.4)	0.492

Data were presented as median (IQR).

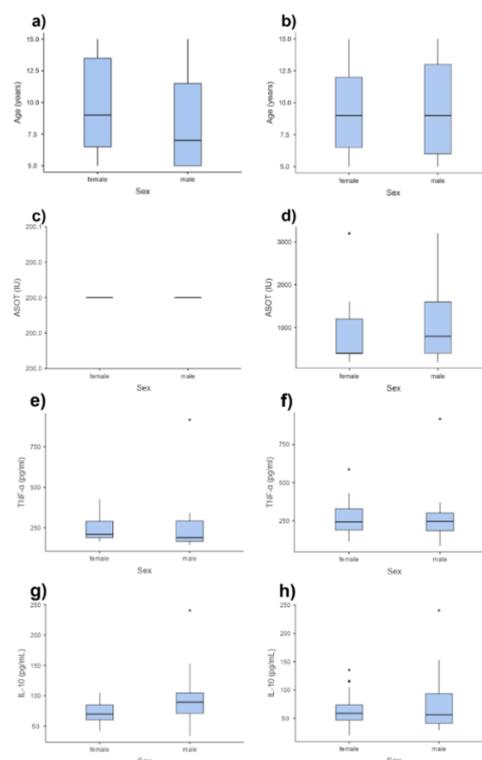


Figure 1. Concentrations of Immunological Markers in Study Participants by Gender

In [Figure 1 a, c, e, and g](#) the results of age, ASO titer, TNF- $\alpha$ , and IL-10 in the control group are shown, respectively; while the results of age, ASOT, TNF- $\alpha$ , and IL-10 are shown in [Figure 1 b, d, f, and h](#) respectively.

## Discussion

Antistreptolysin O titer (ASO) is a blood serum level of antistreptolysin O antibodies used to diagnose streptococcal infections or confirm previous exposure to streptococci. The ASOT guides antibiotic treatment and aids in the diagnosis of scarlet fever, rheumatic fever, and post-infectious glomerulonephritis. A positive streptococcal antibody test can indicate recent group A, C, and G streptococcal infection and support the diagnosis of post-infection complications. The antibodies level rises in 1-3 weeks after infection, peaks in 3-5 weeks, and returns to insignificant levels after 6-12 months. Rising titers over time are symptomatic of infection [13]. This study supports [14] finding that gender is not a risk factor for infection, but differs from [15] finding that females are more susceptible and respond to infection in a 3:1 ratio.

The variables of tumor necrosis factor alpha and interleukin 10 are consistent with these studies. The study found that patients with chronic and acute tonsillitis had higher serum levels of tumor necrosis factor-alpha. TNF- $\alpha$  stimulates the arterial lining and increases permeability, leading to increased immune cell entrance, complement proteins, and IgG and IgA levels. It also promotes fluid outflow to lymph nodes [16]. IL-10, a key regulator of innate immunity, has been demonstrated to have anti-inflammatory properties and play a role in infection, according to multiple research. According to [12], the reaction to microbes and their products triggers the production of IL-10, which is a key factor in pathogenesis [17].

## 5. CONCLUSION

The study found that *Streptococcus pyogenes* is the main reason of chronic tonsillitis. Immunologic markers found that TNF- $\alpha$  is not a sensitive marker for chronic tonsillitis, IL-10 is a sensitive marker, and ASOT index is a marker for infection severity.

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## Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Nebras Ayad Mohammed	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
Sundus Jassim Muhammad		✓		✓		✓		✓	✓			✓		✓
Abdulwadood Shakir Mahmood Alsofi	✓	✓		✓	✓		✓			✓		✓	✓	

C: Conceptualization

M: Methodology

So: Software

Va: Validation

Fo: Formal Analysis

I: Investigation

R: Resources

D: Data Curation

O: Writing- Original Draft

E: Writing- Review & Editing

Vi: Visualization

Su: Supervision

P: Project Administration

Fu: Funding Acquisition

## Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

### Ethical Approval

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and approved by the relevant institutional authorities.

### Data Availability

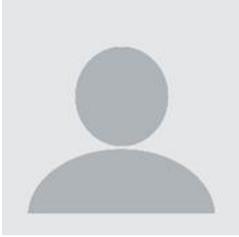
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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