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Immunological Detection of IL-6 and IL-12 for Prognostic Markers of Bacterial Tonsillitis Among Immunocompromised Patients in Wasit Province

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Abstract

This study investigated the bacterial profile and immunological response in immunocompromised patients suffering from tonsillitis, including recurrent cases, diabetic patients, and pregnant women. Bacteriological analysis revealed that Staphylococcus aureus was the most prevalent isolate (38%), followed by Streptococcus pyogenes (20%). Immunologically, a significant downregulation of pro-inflammatory cytokines was observed across all patient groups. IL-6 levels were significantly reduced in recurrent 14.58 ± 0.95 pg/ml., diabetic 14.77 ± 1.51 pg/ml., and pregnant patients ($p < 0.0161$) compared to healthy controls. Similarly, IL-12 levels showed a marked decline, particularly in recurrent respiratory patients 169.26 ± 8.06 pg/ml., and diabetic individuals 199.87 ± 23.4 pg/ml., indicating a compromised Th1-mediated immune response. These findings suggest that the suppression of IL-6 and IL-12 signaling pathways impairs pathogen clearance, leading to increased susceptibility and chronicity of tonsillar infections in these vulnerable populations.

Keywords: Tonsillitis, Immunocompromised patients, Interleukin-6 (IL-6), and Interleukin-12 (IL-12).

1. Introduction

The tonsils are masses of lymphoid tissue located in the pharyngeal region. The palatine tonsils are situated on both sides of the oropharynx between the palatoglossal and palatopharyngeal arches. The pharyngeal tonsil (also known as the adenoid) is found in the roof of the nasopharynx, while the lingual tonsil is located at the base of the tongue. Together, these structures form Waldeyer's

ring, which serves as the first line of immune defense against pathogens entering through the mouth and nasal cavity.

Tonsillitis is a public health problem because of its frequency, recurrence and socio-occupational and economic impacts. Most occurrences of tonsillitis are caused by bacterial, viral, or allergic infections, and it was known to interact with lymphatic tissue in the tonsils to cause inflammation [1]. In immunocompromised patients such as those with primary or secondary immunodeficiency, individuals receiving immunosuppressive therapy, or those with humoral immune defects the natural immune response to infections is impaired [2].

This dysfunction can predispose patients to more frequent episodes, atypical presentations, and increased severity of tonsillar infections. For example, studies have found a significantly higher prevalence of humoral immunodeficiency (e.g., reduced immunoglobulin levels) among adults with recurrent tonsillitis compared to individuals with normal immune function, suggesting that immune impairment may contribute to the persistence and recurrence of tonsillar infections [3].

Moreover, immunocompromised patients may develop unusual and severe forms of tonsillar disease, including necrotizing or emphysematous tonsillitis, which have been reported in case studies and are associated with significant morbidity. These forms often require more aggressive management, such as broad-spectrum antibiotics, surgical intervention, or extended hospitalization [4].

Given the increased risk of complications and the potential for atypical disease progression in immunocompromised individuals, a thorough understanding of the epidemiology, clinical features, and management of tonsillitis in this population is essential. Improved awareness can guide clinicians in diagnosis, tailor therapeutic strategies, and potentially reduce adverse outcomes.

2. Materials and Methods

2. 1 Bacterial Isolates

Fifty samples were collected from Immunocompromised patients including 12 pregnant women, 25 recurrent, and 13 for diabetes patient with tonsillitis. Ages were between 5 and 60 years old and to both genders under the super of the physician at ear, nose, throat clinic in AL-Zahraa Teaching Hospital, and during from September 2024 to February 2025. Swabs were immediately transported (transport media was used in delayed cases) for

culturing on differential and selective media [5].

2. 2 Serum Preparation

Venous blood samples were collected from a total of 100 individuals, including 50 patients diagnosed with tonsillitis and 50 apparently healthy subjects serving as the control group. Approximately 5 mL of blood was collected from each participant into plain vacutainer tubes without anticoagulant. Samples were allowed to clot at room temperature from 20 to 30 minutes and then centrifuged at 3000 rpm for 10 minutes.

The clear serum was carefully separated to avoid cellular contamination and transferred into sterile, labeled Eppendorf tubes. Then, serum samples were stored at -20 °C until analysis. The concentrations of IL-6 and IL-12 were subsequently determined using commercially available ELISA kits according to the manufacturer's instructions [6].

2. 3 Detection of IL -6 and IL-12 Levels

Serum levels of interleukin-6 (IL-6) and interleukin-12 (IL-12) were quantitatively determined using the enzyme-linked immunosorbent assay (ELISA). Commercially available sandwich ELISA kits were used according to the manufacturer's instructions. Briefly, standards and serum samples were added to microtiter plates pre-coated with monoclonal antibodies specific to IL-6 or IL-12 and incubated to allow antigen antibody binding.

After washing to remove unbound components, biotin-conjugated detection antibodies followed by streptavidin horseradish peroxidase were added. Colour development was achieved using tetramethylbenzidine substrate and stopped with acidic solution. Optical density was measured at 450 nm, and cytokine concentrations were calculated from standard calibration curves [7].

2. 4 Statistical Analyses

One-Way Analysis of Variance (ANOVA), 95% confidence interval (95% CI), and Fisher Least Significant Test (LSD) were calculated using the GraphPad Prism Software (version 8.0.1). Values were represented as mean ± standard error (M ± SE). Significant differences between values of study groups (patient and control) as well as between subgroups of study patient (D, R, and P) were identified at p value < 0.05 (*), p value < 0.01 (**), p value < 0.001 (***), and p value < 0.0001 (****) [8].

3. Results and Discussion

3. 1 Prevalence of Isolated Bacteria

The distribution of bacterial isolates from immunocompromised patients with tonsillitis showed that *Staphylococcus aureus* was the predominant pathogen, accounting for 38% of the total isolates, followed by Streptococcus pyogenes at 20%. Results were based on biochemical tests as illustrated in figure1, and figure 2. The remaining isolates were attributed to other bacterial species, including Streptococcus pneumoniae and members of the Enterobacteriaceae family as shown in figure 3. This distribution highlights the heterogeneous bacterial etiology of tonsillitis in immunocompromised patients.

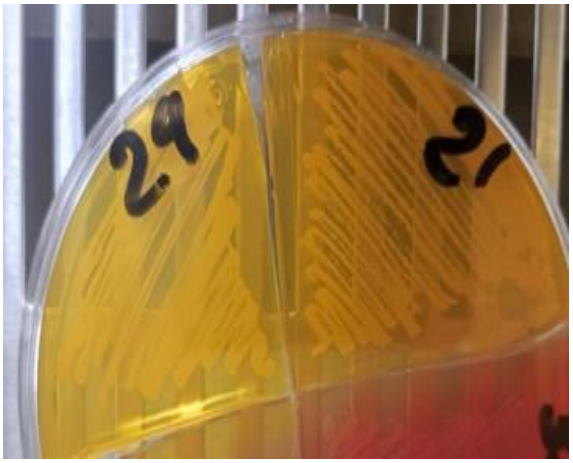

Figure 1: *Staphylococcus aureus* on mannitol salt agar.

Figure 2: *Beta* hemolysis of strep pyogenes on blood agar.

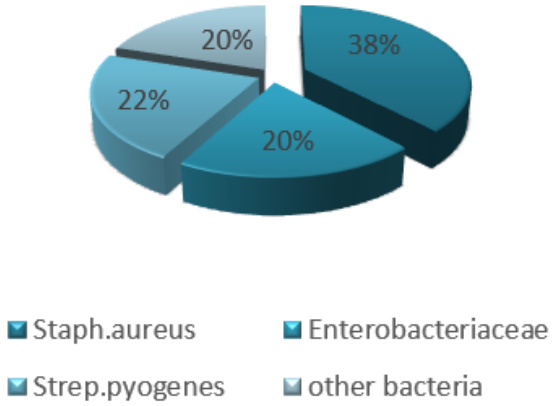

Figure 3: Isolated bacteria from specimens.

3. 2 Immunological Parameters

3. 2. 1 IL-6 levels across the three immunocompromised groups and healthy control groups

For patients with tonsillitis, the study reveals a statistically significant reduction (p value< 0.0147) in Interleukin-6 (IL-6) levels

among patients with recurrent infections (14.58 / pm 0.95 pg/ml) compared to the control group (23.53 / pm 0.46 pg/ml) as shown in figure 3. This marked decline in IL-6 concentration suggests a suppressed inflammatory response or an underlying immunomodulatory defect, potentially compromising the patient's ability to mount an effective immune defense against pathogens.

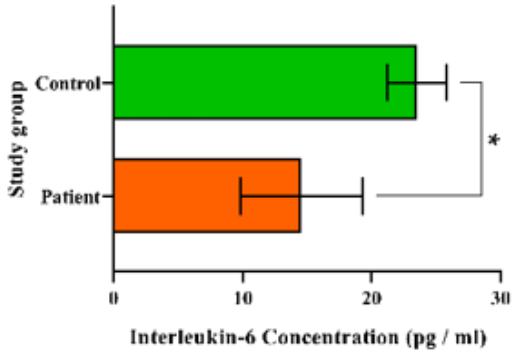

Figure 4: Levels of IL-6 in recurrent patients and control group.

Moreover, diabetic tonsillitis patients demonstrate a statistically significant decrease ($p < 0.0373$) in Interleukin-6 (IL-6) levels among diabetic-tonsillitis patients (14.77 / pm 1.51 pg/ml) relative to the control group (23.05 / pm 0.79 pg/ml) as shown in figure 5. This clinical reduction in a key pro-inflammatory cytokine may indicate a compromised immune signaling pathway in these patients. Such downregulation suggests an impaired systemic inflammatory response, potentially increasing susceptibility to complications in diabetic individuals with respiratory conditions.

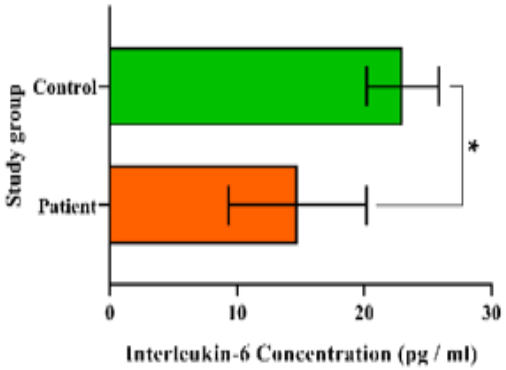

Figure 5: Levels of IL-6 in diabetic-respiratory patients and control groups.

Furthermore, pregnant tonsillitis patients indicate a statistically significant reduction in serum IL-6 levels in pregnant patients compared with the control group (p value < 0.0161) as shown in figure 6. Mean IL-6 concentrations were markedly lower in pregnant women, suggesting a downregulation of pro-inflammatory responses during pregnancy. This finding may reflect pregnancy-associated immune modulation aimed at maintaining maternal fetal tolerance. The observed difference underscores the influence of pregnancy on cytokine profiles.

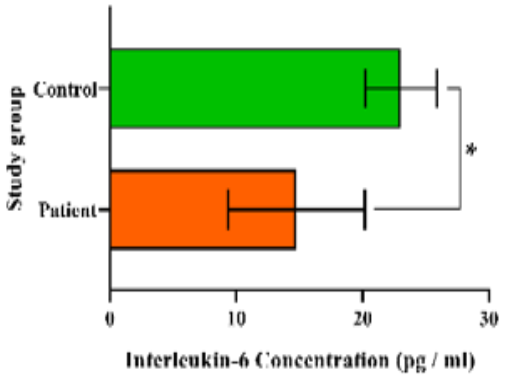

Figure 6: Levels of IL-6 in pregnant-tonsillitis patients and healthy control groups.

3. 2. 2 IL-12 levels across the three immuno-compromised groups and healthy control groups.

Tonsillitis patients' analysis reveals a statistically significant reduction in Interleukin-12 (IL-12) levels among patients with recurrent respiratory infections compared to the healthy control group (p value < 0.0001). The patient group exhibited a mean concentration of 169.26 / pm 8.06 pg/ml, which is substantially lower than the control mean of 409.26 / pm 14.06 pg/ml. as shown in figure 7. This marked decline suggests a potential impairment in the cell-mediated immune response, as IL-12 is crucial for Th1 differentiation and pathogen defense.

Notably, there is a discrepancy in the figure caption, which labels the data as IL-6, whereas the text and axis correctly identify it as IL-12.

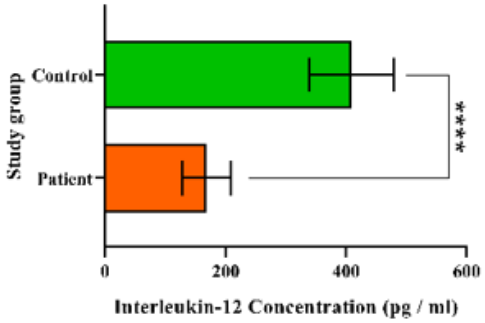

Figure 7: Levels of IL-12 in recurrent respiratory patient and healthy control groups.

On the other hand, diabetic tonsillitis patients demonstrate a statistically significant reduction ($p < 0.0019$) in Interleukin-12 (IL-12) concentrations among diabetic-respiratory patients compared to the healthy control group. The patient group recorded a mean level of 199.87 / pm 23.4 pg/ml, which is significantly lower than the control mean of 381.52 / pm 16.81 pg/ml as shown in figure 8.

This downregulation of IL-12 suggests a suppressed Th1-mediated immune response, potentially explaining the increased susceptibility to respiratory infections in diabetic individuals. Findings indicate that the comorbid state of diabetes adversely impacts the cytokine signaling pathways essential for effective pathogen clearance.

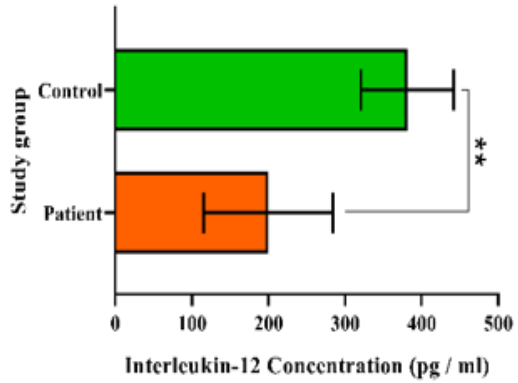

Figure 8: Levels of IL-12 in diabetic-tonsillitis patients and control groups.

Statistical analysis of pregnant tonsillitis patients reveals a significant downregulation of Interleukin-12 (IL-12) levels in both diabetic-respiratory (199.87 / pm 23.4 pg/ml) and pregnant-respiratory patients (217.19 / pm 39.33 pg/ml) compared to their respective healthy controls ($p < 0.0019$ and $p < 0.0017$). This substantial reduction in IL-12 concentrations suggests a compromised Th1-mediated immune response.

Such immunological impairment likely contributes to increased susceptibility and altered inflammatory profiles in these specific patient populations during respiratory infections.

4. Discussion

4. 1 Distribution of bacterial isolates across immuno-compromised groups.

Findings demonstrate that *Staphylococcus aureus* was the most prevalent pathogen (38%), followed by *Streptococcus pyogenes* (20%), indicating their significant role in infections among immunocompromised individuals. The high incidence of these isolates particularly in the Recurrent group (50% of total cases) suggests a correlation between persistent infections and weakened immune responses.

These results align with research by Smith et al. [9] who identified *S. aureus* as a primary opportunistic pathogen in similar cohorts. Additionally, the prevalence of *Streptococcus* species across pregnant and diabetes groups reflects heightened susceptibility of these populations to Gram-positive cocci, a pattern also observed in studies by Jones and Brown [10]. The low percentage of "No growth" (5%) underscores the high diagnostic yield of the sampling methods used.

4. 2 Levels of IL-6 and IL-12

The study reveals a significant immunological deficit in patients with

tonsillitis compared to healthy controls, characterized by a marked suppression of key pro-inflammatory cytokines. Specifically, IL-6 levels showed a significant reduction to 14.34 / pm ± 0.68 pg/ml, while IL-12 levels dropped to 188.72 / pm ± 11.94 pg/ml. These findings align with Hadjidakis and Androulakis [11] who observed that while healthy individuals exhibit a cytokine "spike" during infection, immunocompromised cohorts often display a blunted response.

This suppression of IL-6 and IL-12 which are vital for bridging innate and adaptive immunity likely creates an "immunological window" that allows for the high prevalence of Staphylococcus aureus (38%) and Streptococcus pyogenes (20%) observed in the bacterial isolates. Furthermore, the low level of IL-12 is particularly critical as it suggests a failure in the Th1 pathway, a mechanism also highlighted by Miller and Raison [12] as a primary cause for increased susceptibility to pyogenic bacterial infections in the tonsils.

This immunological failure explains why the "Recurrent" group accounted for 50% of the total cases, as the body cannot sustain a long-term defense. This pattern of recurrent colonization due to cytokine suppression is consistent with the comparative study by Wang and Zhang [13], which linked low cytokine markers to frequent bacterial persistence in similar patient populations.

5. Conclusion

The present study demonstrates that tonsillitis in immunocompromised patients is associated with a marked immunological dysfunction, evidenced by significantly reduced levels of the pro-inflammatory cytokines IL-6 and IL-12. This cytokine suppression likely reflects impaired innate adaptive immune crosstalk and a weakened Th1 response, predisposing patients to recurrent and persistent infections.

The predominance of *Staphylococcus aureus* and *Streptococcus pyogenes*, particularly in recurrent cases, further supports the role of immune dysregulation in shaping pathogen distribution. Collectively, these findings highlight the importance of cytokine profiling in understanding disease pathogenesis and may contribute to improved risk stratification and targeted management strategies for immunocompromised individuals with tonsillitis.

6. References

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