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The role of IL-10 ,TNF-α(levels) and Anti-streptolsinas a Biomarkers in Pre-diagnosis of Recurrent Tonsillitis.

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البصمة المناعية مميزة لإنترلوكين-10/عامل نخر الورم ألفا واستجابة مضادات الأوليغونوكليوتيدات في التهاب اللوزتين المتكرر

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ABSTRACT

Background:
Background Tonsillitis in children is a widespread otolaryngology condition that is typically associated with impaired immunity and frequent epidemics of Streptococcus species.

Methods: Between September and December 2024a case-control study was done in the Ramadi Teaching Hospital, Iraq. The study involved thirty age- and sex-matched controls who were healthy and sixty children with a proven clinical diagnosis of recurrent tonsillitis. The venous blood sample was collected on all the participants. ASO titers were quantified using the latex agglutination test and TNF-a and IL-10 levels were identified using (ELISA). Statistical analysis was used to compare the immunological properties of the two groups.

Result: Children with recurrent tonsillitis had higher ASO titers than controls (1250 vs. 750 IU), with no significant gender difference ($p > 0.05$), indicating streptococcal infection .IL-10 levels were lower in patients compared to controls (43.4–50.5 vs. 65.9–85.9 pg/mL; $p < 0.05$), reflecting impaired anti-inflammatory response .

Conclusion: The results indicate that tonsillitis in children that happens repeatedly is associated with high ASO levels and low IL-10 levels indicating that there may be an impaired immune regulation, which may predispose children to the persistence of the disease. Immunological assessment could help in the management approach and guide the creation of Immunomodulatory approaches that are specific.

Key word: Chronic tonsillitis, IL-10, TNF-α,ASO, *Streptococcus pyogenes*.

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INTRODUCTION

Four sets of anatomically differentiated tonsils exist to form the Waldeyer ring, and every set of tonsils is in a favorable anatomic location to trap the antigen while promoting an immune response [1,2]. The parapharyngeal space is situated between the palatine tonsils, which is where the consequence of secondary infections or complications is most likely to occur particularly when there are chronic or supportive tonsils. Therefore, when conducting surgical procedures such as tonsillectomy or peritonsillar abscess drainage, one should possess unambiguous knowledge of the anatomy of surgical operations in the tonsillar area, and spaces surrounding tonsillar area [3,4]. It may be caused etiologically by viral agents, including adenoviruses, rhinoviruses, Epstein-Barr virus among others or by bacterial pathogens most notably Streptococcus pyogenes (Group A paBH streptococcus), which continues to be the most important causative agent of acute bacterial tonsillitis [4,5,7]. Pathogenicity of Streptococcus pyogenes is mediated by numerous virulence factors which lead to vigorous immune reactions and tissue injury. Anti-streptolysin O (ASO) titers are immunological determinants of recent streptococcal infection often applied in the clinical diagnosis [6,7]. Moreover, the same can be said about the occurrence of enteric pathogens in tonsillar culture, i.e. Escherichia coli, which is an indication of fecal-oral transmission, particularly, in populations with poor hygienic habits. One of the factors that is a great cause of concern in dealing with tonsillitis is the increase in prevalence of multidrug resistance (MDR) strains within the microbial flora that are sited in the tonsillar crypts. The inappropriate use of antibiotics, self-medication as well as failing to follow the existing standards of antimicrobial stewardship are closely associated with the occurrence of these strains. This opposition is not only a complicating factor in therapeutic decision-making, but it also predisposes to failure of treatment, relapse, and development of such complications as peritonsillar abscess or rheumatic fever [8]. The aim of the study, evaluate the immunological and microbial profile in children with recurrent tonsillitis by measuring ASO titers, IL-10, and TNF-α levels, and comparing them with healthy controls. To assess the role of these biomarkers in inflammation, immune regulation, and their association with streptococcal infection in recurrent tonsillitis.

MATERIALS AND METHODS

Study Design and Setting

The case control study will be carried out in the ENT Department of Ramadi Teaching Hospital in Al-Anbar, Iraq, in September-December 2024. The Scientific and Ethical Committee of the College of Science of the University of Anbar approved the study.

Study Population

The study included 60 (patient and control) aged 4–14 years of both sexes, comprising children clinically diagnosed with recurrent tonsillitis according to established ENT criteria, along with 30 age- and sex-matched healthy controls. Children with immunodeficiency disorders, recent antibiotic use within the previous two weeks, or any acute illness at the time of sampling were excluded.

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Collection and Processing

Patients had their throat swabs taken. Swabs were inoculated with transport medium in late-stage cases of infections (long suffering or use of antibiotics) and transported to preserve the viability of the bacteria. Healthy individuals were also taken as controls and swabs were collected under sterile conditions and transported to the media to prevent any form of contamination. All the samples were immediately taken to the laboratory. Bacteria could be separated using a variety of different media, e.g., with the help of differential media (blood agar) to differentiate them depending on the pattern of hemolysis, or selective media (e.g., MacConkey agar) to enhance the growth of particular pathogens, at the expense of normal flora.

Laboratory Reagents

The level of serum anti-streptolysin O (ASO) was measured with the help of the latex agglutination kit (Atlas Medical, Cambridge, UK), which has included latex particles coated with purified streptolysin O antigen, positive and negative control serum, glycine-saline buffer, and test cards of qualitative and semi-quantitative detection.Serum interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-a) were quantified using human ELISA These kits simply consist of pre-prepared 96-well microplates of monoclonal antibodies specific to the target cytokine, biotin-conjugated detection antibodies, recombinant human standards, with dilution buffers, streptavidin-HRP conjugate, TMB substrate solution, acidic stop solution, and concentrated wash buffer, which will allow making accurate and reproducible measurements .

STATISTICAL ANALYSIS

The analysis of the data was conducted with the help of SPSS version 25.0. In the case of continuous variables, mean values and their standard deviations (mean ±SD) have been used as the results. Frequencies and respective percentages were used to describe categorical variables. An Independent-samples t-test was used to determine whether there are differences between groups. The statistical relevance was determined at a level of p less than 0.05.

RESULTS

Each of the participants was swabbed on their throats to obtain their microbiology cultures. It was reported that 65 percent of the samples of patients had bacterial development. Streptococcus pyogenes (40%), followed by Staphylococcus aureus (20%) were the largest percent of isolated pathogen. Other isolates such as Escherichia coli and Haemophilus influenzae were included about 5-10 per cent of positive cultures and consisted predominantly of normal flora, such as Streptococcus mitis and Staphylococcus epidermidis . These findings inferred that the microbial profile of tonsil swabs in healthy patients was very differentfrom that in tonsillitis patients and healthy controls, implying the implication of the use of some bacterial pathogens in the pathogenesis of tonsillitis.

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Comparison Between Tonsillitis Patients and the Control Group According to Gender and Age:

All participants had their throats swabbed. The group of patients had a 65% bacterial growth. The most frequently isolated pathogens were Streptococcus pyogenes (40 percent), and Staphylococcus aureus (20 percent). Others, Escherichia coli and Haemophilus influenzae, were also identified to cause approximately 5-10% of positive cultures; these ones were predominantly normal flora, such as Streptococcus mitis and Staphylococcus epidermidis. These findings indicate a substantial difference between the microbial profile of tonsillar swabs of tonsillitis and healthy controls, which prompts the role of some pathogenic bacteria in the pathogenesis of the disease to play a significant role, show figure 1.

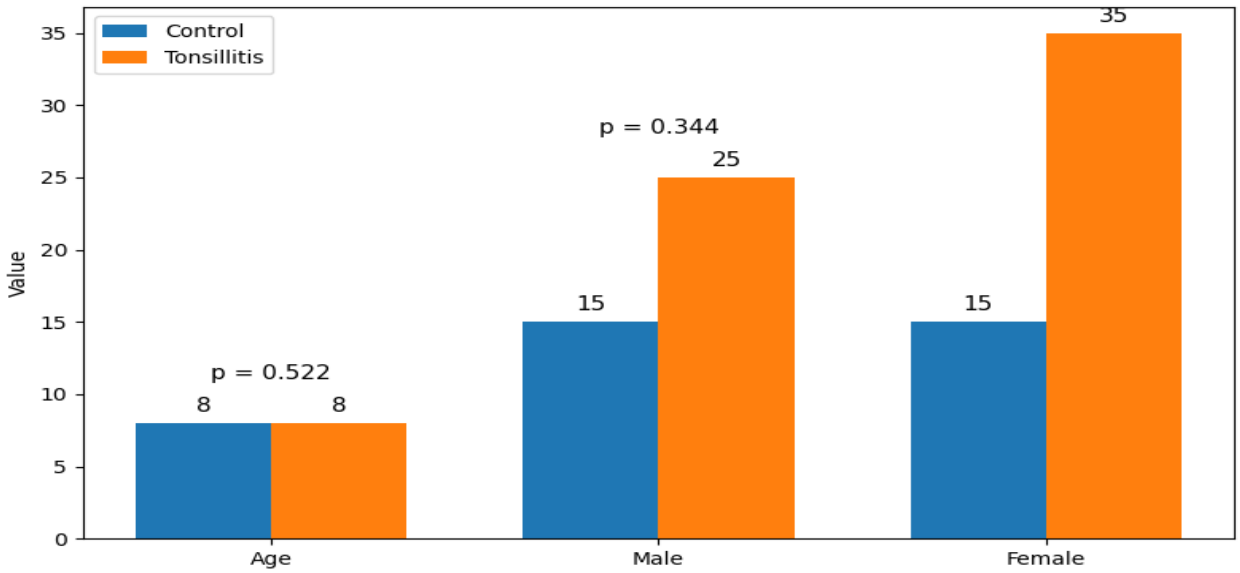

Figure 1 Demographic Comparison of Tonsillitis Patients and Healthy Controls Based on Age and Gender

The Levels of Serum IL-10 Concentration by Gender in Control and Tonsillitis Groups

The means of IL-10 concentration in males and females were compared in the control and tonsillitis group (Figure 2) to widen gender-related disparities regarding the expression of IL-10. The serum levels of IL-10 concentration were higher in males (85.9 pg/mL) compared to females (65.9 pg/mL). On the other hand, in the tonsillitis one, females none the less had a small higher IL-10 (50.5 pg/mL) than the males (43.4 pg/mL). Such an inverted pattern may indicate sex differences in immune regulations in the condition of chronic stress of inflammation. Nevertheless, as is the case with the control group, such differences were not statistically significant (P = 0.388).It means that even though the differences between genders in each sample group are not zero, they are not that significant to imply a high level of gender-specific immunological pattern. Notably, the level of IL-10 in both male and female tonsillitis patients was significantly lower than the controls of both sexes, which supports the concept of the loss of regulatory cytokine functions in the disease condition.

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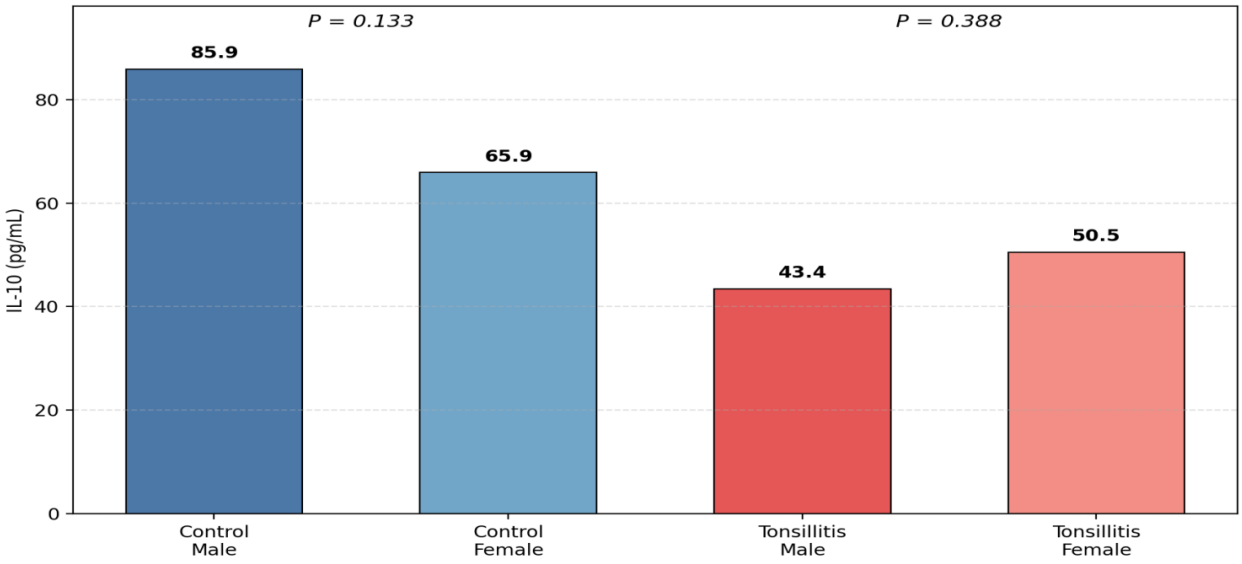

Figure 2 IL-10 Concentration by Gender in Control and Tonsillitis Groups

The levels of Serum TNF-α by Gender in Control and Tonsillitis Groups

The comparative analysis of serum TNF-α levels between males and females in both the control and tonsillitis groups revealed an overall increase in TNF-α concentrations among patients with recurrent tonsillitis. In the control group, males exhibited a mean TNF-α level of 205 pg/mL, while females showed a slightly lower mean value of 187 pg/mL. TNF-α levels were elevated in both sexes compared to their counterparts in the control group. Male patients demonstrated a mean level of 255 pg/mL, whereas female patients exhibited a similar mean value of 257 pg/mL. The difference between sexes in the patient group was minimal and statistically insignificant, as reflected by a P-value of 0.543. These results suggest that TNF-α plays a role in the inflammatory process associated with recurrent tonsillitis, with consistently higher levels observed in affected individuals regardless of gender. However, the lack of statistically significant differences between males and females within each group implies that gender does not have a strong influence on the expression of this cytokine under either normal or diseased conditions(figure 3).

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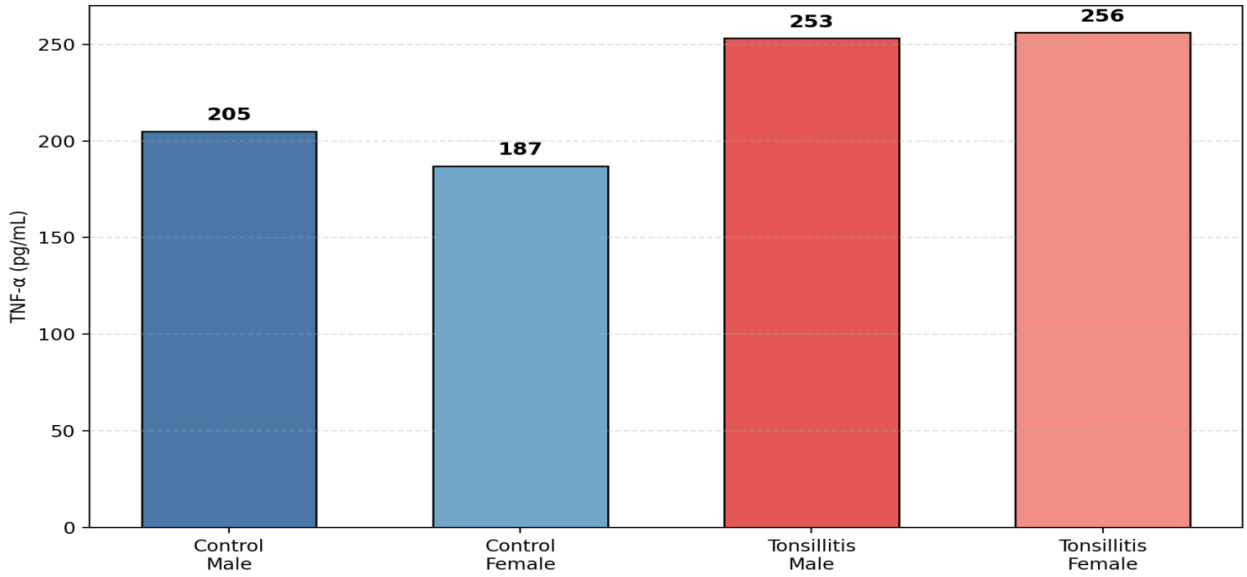

Figure 3 serum TNF-α Levels by Gender in Control and Tonsillitis Groups

The levels of Serum ASO titer by Gender in Control and Tonsillitis Groups

The median ASO level in the tonsillitis group was significantly high, at 413 IU. On sex analysis, the mean ASO level of the male patients was significantly higher at 1250 IU as opposed to the female patients at 750 IU. Such findings demonstrate that there is a significant variation in the antibody response between the male and female patients in the tonsillitis group. The statistical test proved the fact that no significant gender-based difference was observed and the P-value was 0.888. Conversely, the tonsillitis group had high ASO titers between males and females, where higher values were observed in males. The average ASO of the male patients was 1250 IU as opposed to 750 IU in females. Although there is a numerical difference in this comparison, the difference was not statistically significant, as the P-value of 0.455 shows, indicating that ASO titers are significantly higher in patients with recurrent tonsillitis which proves this hypothesis of a streptococcal-driven immunological response. Nevertheless, the male and female differences in either group could not be statistically significant showing that the ASO production is not highly affected by gender in either healthy or disease states(figure 4).

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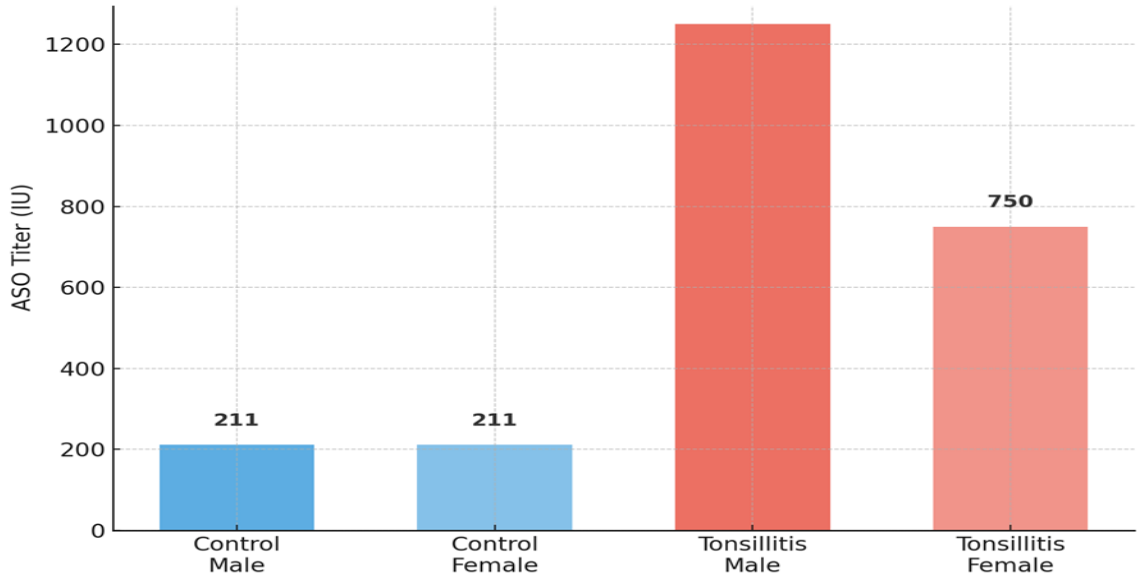

Figure 4 Serum ASO titer by Gender in Control and Tonsillitis Groups

Integrated Analysis of IL-10, TNF-α, and ASO Titer Across Study Groups

Combined IL-10, TNF-a, and ASO titer analysis indicates an evident state of the immune imbalance in patients with recurrent tonsillitis. The anti-inflammatory IL-10 levels were greatly lower in the patients as compared to controls particularly the male counterparts. Contrary to that, TNF-a, a pro-inflammatory cytokine, was high in both males and females, which shows that there was an ongoing presence of an inflammatory response. Also, the ASO titers were significantly elevated in the tonsillitis group especially in males, implying recent or chronic exposure to streptococcal infections. The results indicate a negative correlation between IL-10 and TNF-a and ASO titers. These data corroborate some pattern of impaired immune regulation (low IL-10) and increased inflammation (high TNF-a) and streptococcal activation (high ASO) and contribute to the chronicity of tonsillitis with males having a stronger response.

The present study revealed in 65 of 100 throat swabs taken of patients with tonsillitis that there was growth of bacteria with Streptococcus pyogenes (40 percent) as the most prevalent bacterium after which Staphylococcus aureus bacteria were detected in 20 percent of cases, which were earlier reported to be the second most common pathogen in acute tonsillitis [9-11]. Conversely, commensal flora such as Streptococcus mitis and Staphylococcus epidermidis were identified to be the only organisms in the healthy controls, which aligns with the descriptions of the normal flora on the pharynx of healthy people [12-14]. Diversities between the studies could be partly explained by the variation in the methodologies such as using surface swabs and tonsillar core samples and applying the standard culture methods versus molecular diagnostic methods [15-17]. The earlier researchers have stated that molecular diagnostics is capable of identifying active infection, as well as colonization of bacteria which may be the reason behind the difference portrayed by various studies [18,19]. The sophisticated studies that involve culture

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and molecular in the future can thus enhance the distinction between actual infection and colonization in children of school going age (5-15 years), which is in line with the global epidemiological trends [20-22]. There have been some reports that indicate a slight male predominance in cases of tonsillitis [23,24], however, current research did not find any statistically significant differences between males and females, which is in agreement with other researchers that found close to equal male-to-female ratios [25]. This study also reduced the possible confounding factors because it balanced the demographic variables between cases and controls and provided greater validity of the microbiological comparisons [26,27]. Interestingly, patients with tonsillitis showed a remarkable decrease in the level of IL-10 in comparison with their healthy counterparts regardless of their gender [28-30]. This observation is in line with other studies that have highlighted the importance of the dysregulation of regulatory cytokines to perpetuate inflammation and destabilize immune tolerance in tonsillar tissues [31-33]. Despite the fact that minimal sex-specific differences in the expression of IL-10 have been observed [34,35], the state of the disease seems to be the major determinant in the cytokine expression levels. Conversely, the concentration of TNF-a was much higher in the patients, which indicates a more pro-inflammatory condition. This observation is associated with previous findings that the expression of TNF-a is correlated with the recurrence of tonsillitis and tonsillar hypertrophy [36-38]. The lack of gender difference in the TNF-an expression is also alignedwith the literature that has indicated the presence of little impact of sex hormones on the production of TNF-a among child subjects [39-41]. Moreover, the presence of substantially high levels of antistreptolysin O (ASO) in patients confirms the possibility of the recent or repeated exposure to the group A streptococcal infections, which is consistent with the definitions of the existing diagnostic systems [42-44]. The simultaneous IL-10/TNF-a/ ASO titer evaluation demonstrates the evident situation of immune inbalance in the case of recurrent tonsillitis, with the levels of anti-inflammatory IL-10 being significantly lower with superior levels of TNF-a being present in all the genders, which shows the persistence of inflammation. There was also a high ASO titer in the tonsillitis group with high rates especially in the males which may indicate recurrence of streptococcal infection. In addition, there was a negative association between IL-10 and TNF-a as well as ASO titers, which showed poor immune regulation (low IL-10), inflammatory response (high TNF-a) and streptococcal immune stimulation (high ASO), which could lead to chronic tonsillitis (45,46).

CONCLUSION

These results of the current study indicate that the children who had repeated cases of tonsillitis display profound immunologic imbalance not only high levels of anti streptolysin O (ASO) levels, but also low levels of interleukin-10 (IL -10) levels signifying consistent exposure to streptococcal infection and inability to respond to the infection. In its turn, the concentrations of tumor necrosis factor-alpha (TNF- alpha), however, did not differ between the patients and the healthy controls, yet it indicates that the systemic pro-inflammatory activation was not the most significant factor of the recurrence. These observations predetermine the need to explore

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immunoregulatory phenotypes in the patients with recurring tonsillar infection and determine the potential of individual immunomodulatory treatment in the improvement of the disease process and the decrease of the frequency.

Practical Recommendations

Children who experience recurring cases of tonsillitis ought to be screened frequently with regard to the levels of IL-10 and ASO to determine the existence of immune imbalances and new streptococcal infections. Cytidine modification involving immunomodulatory effects that boost ant-inflammatory actions, especially IL-10 might minimize recurrence. Monitoring of ASO, streptococcal infections is detected early to lead to the on-time treatment. The personalization of management plans should be dependent on immune profiles, and not on symptoms. It is important to raise awareness of healthcare providers and caregivers with respect to the role of immune dysregulation. It is suggested that their findings should be further investigated by carrying out research to establish the same and the implications of such research.

1-Ethical Approval:

1. Ethical Approval: The Local Ethics Committee approved the study, No. 178, of the College of Science, University of Anbar, on December 1, 2024, and the study adhered to the standards of good clinical practice.
2. Consent: All cases studied signed informed consent.
3. Conflicts of Interest The authors declare that they have no conflicts of interest.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

الخلفية: يُعدّ التهاب اللوزتين لدى الأطفال من الحالات الشائعة في اختصاص الأنف والأذن والحنجرة، ويرتبط غالبًا بضعف الاستجابة المناعية وتكرار العدوى بالمكورات العقدية. **الطرق:** أُجريت دراسة حالة–شاهد في مستشفى الرمادي التعليمي/العراق خلال الفترة من سبتمبر إلى ديسمبر 2024. شملت الدراسة 30 طفلًا سليمًا مماثلين في العمر والجنس كمجموعة ضابطة، و60 طفلًا مشخصين سريريًا بالتهاب اللوزتين المتكرر. جُمعت عينات دم وريدي من جميع المشاركين. تم قياس عيارات الأجسام المضادة للمكورات العقدية (ASO) باستخدام اختبار التراص اللاتكس، بينما حُددت تراكيز عامل نخر الورم ألفا (TNF–α) والإنترلوكين–10 (IL–10) باستخدام المقايسة الامتصاصية المناعية للإنزيم المرتبط، مع اعتماد تراكيز المعايير القياسية في المعايرة. أُجري التحليل الإحصائي لمقارنة الخصائص المناعية بين المجموعتين.

النتائج: أظهر الأطفال المصابون بالتهاب اللوزتين المتكرر ارتفاعًا في عيارات ASO مقارنةً بالمجموعة الضابطة (1250 مقابل 750 وحدة دولية)، دون وجود فرق معنوي بين الجنسين($p > 0.05$) ، مما يشير إلى وجود عدوى بالمكورات العقدية. كما انخفضت تراكيز الإنترلوكين–10 لدى المرضى مقارنةً بالأصحاء (43.4–50.5 مقابل 9.65–85.9 بيكوغرام/مل؛ $p < 0.05$) ، مما يعكس ضعف الاستجابة المضادة للالتهاب.

الخلاصة: تشير النتائج إلى أن التهاب اللوزتين المتكرر لدى الأطفال يرتبط بارتفاع عيارات ASO وانخفاض تراكيز IL–10 ، مما يدل على احتمال وجود خلل في تنظيم الاستجابة المناعية قد يساهم في استمرار المرض. ويمكن أن يسهم التقييم المناعي في تحسين إدارة الحالة وتوجيه تطوير استراتيجيات علاجية قائمة على تعديل المناعة بشكل مخصص.

الكلمات المفتاحية: التهاب اللوزتين المزمن، إنترلوكين–10، عامل نخر الورم ألفا، مضاد للبكتيريا العقدية المقيحة، المكورات العقدية المقيحة