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Role of SAgs Produced by *Staphylococcus aureus*, rs893629 in *TLR2* Gene in Progression of Recurrent Tonsillitis

Rawaa AlChalabi*, Ahmed A. Mhawesh , Ahmed Yaseen AL-Tarboolee , Mohammed H. Wali

Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Jadriya, Baghdad, Iraq

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Abstract

Recurrent tonsillitis is characterized by five or more episodes of severe tonsillitis per year, which significantly impact daily life and normal activities. Superantigens (SAgs) trigger a massive response in T cells, resulting in an overwhelming release of cytokines and rapid T cell growth. Toll like receptor 2 (TLR2) is a substational pattern recognition receptor (PRR) for inflammation and the immune response. This study is designed to detect rs893629 in the *TLR2* gene and genes encoding SAgs A, B and C in *Staphylococcus aureus* isolates. Additionally, the study examines the association between rs893629 and serum level of (TLR2, IFN-γ, MIF and IL-4) as a risk factor for the progression of tonsillitis. 261 patients, 89 healthy volunteers of both sexes, enrolled in this study. The tetra-arm PCR technique was used to detect rs893629, serum levels of TLR2 and cytokines were estimated with sandwich ELISA. Infections of tonsillitis were more common and severe in females than in males. *Streptococcus pyogens* represented the most common bacterial species98(49.2%),69 (34.7%) *Staphylococcus aureus* ,32(16.1 %) *Haemophilus influenzae*. *S. aureus* isolates from tonsillitis patients possesses one or more genes encoded the various types of SAgs, 69 (100%) of the isolates have *SEA*, 57 (82.6%) have *SEB*, and 48 (69.5%) contain *SEC*. The genetic composition and allele frequencies of TLR2 rs893629 exhibited a significant difference between patient and control groups. The heterozygous genotypes GA was predominant among patients with an elevated frequency of allele G, while AA genotypes are dominant among healthy individuals. The results found notable variations between patients with recurrent tonsillitis and healthy controls in serum levels of TLR2, IFN-γ, MIF, and IL-4. In addition, the outcome found a highly significant difference in serum levels of TLR2 protein between patients who have a carrier GA genotype. Our findings indicate that the majority of *S. aureus* isolates produced more than one type of super antigen known as immunostimulatory toxins, that activate enormous numbers of T lymphocytes, significantly associate. The G allele of the *TLR2* gene variant rs893629 is related with an increased risk of recurrent tonsillitis. The G allele is a risk factor that increases the chance of getting the sickness as well as its severity because of its positive link with the disease. High serum levels of TLR2, IFNγ, MIF, and low IL-4 levels are all regarded significant markers that may point to the severity of the illness.

Keywords: Tonsillitis, TLR2, rs893629, Cytokines

*Email: rawaa.alchalabi@nahrainuniv.edu.iq

دور انتاج المكورات العنقودية للمستضدات السطحية وتعدد الاشكال الوراثية (rs893629) في جين مستقبل شبيه التول الثاني في تفاقم التهاب اللوزتين المتكرر

رواء الجلبي، احمد علي مهاوش ،احمد ياسين الطربولي ، محمد حسين والي
قسم التقنيات الحيوية الجزيئية والطبية ، كلية التقنيات الاحيائية ، جامعة النهرين ، العراق ،بغداد ، الجادرية

الخلاصة

التهاب اللوزتين المتكررهو الالتهاب الشديد الذي يصيب اللوزتين لاكثر من خمس مرات في السنة مما يؤثر على الأنشطة العادية حيث تقوم المستضدات السطحية بتنشيط نسبة كبيرة من الخلايا التائية، مما يؤدي إلى تكاثر الخلايا وتحفيز انتاج الستوكينات كما يلعب المستقبل الشبيه بالتول النوع الثاني دور مهم في الالتهاب والاستجابة المناعية. صممت هذه الدراسة للكشف عن تعدد النيوكلوتيدة rs893629 في جين *TLR2* والجينات المشفرة للمستضدات السطحية نوع أ ,ب ,ج في عزلات المكورات العنقودية الذهبية. بالإضافة إلى دراسة الارتباط بين rs893629 والمستوى المصلي لكل منTLR2 و IFN−γ و MIF و IL−4 كعامل خطورة لتطور التهاب اللوزتين.تضمنت الدراسة 261 مريضا، 89 متطوعا أصحاء من كلا الجنسين. تم استخدام تقنية PCR رباعي الذراع للكشف عن rs893629، وتم استخدام ELISA ساندويتش لتقدير مستويات مصل TLR2 والسيتوكينات. النتائج: كانت حالات التهاب اللوزتين أكثر شيوعاً وشدة عند الإناث منها عند الذكور. تمثل المكورات العقدية المقيحة أكثر الأنواع البكتيرية شيوعاً98 (49.2%)تليها المكورات العنقودية الذهبية69 (34.7%) والمستدمية النزلية 32(16.1%). تمتلك عزلات المكورات العنقودية الذهبية من مرضى التهاب اللوزتين واحدًا أو أكثر من الجينات المشفرة للمستضد السطحي بأنواعه المختلفة، 69 (100٪) من العزلات لديها *SEA*،(82.6) 57٪ لديها *SEB*، و 48 (69.5٪) تحتوي على *SEC*. أظهر التركيب الجيني وترددات الأليلات لـ *TLR2* فيrs893629 فرقًا كبيرًا بين المرضى والاصحاء حيث مثل النمط الجيني المتغاير GA النمط السائد بين المرضى الذين لديهم تكرار مرتفع للأليل G، في حين أن النمط الجيني المتماثلAA هو النمط السائد بين الاصحاء. كما كشفت النتائج عن وجود فروق ذات دلالة إحصائية بين مرضى التهاب اللوزتين المتكرر والأشخاص الأصحاء في مستويات TLR2 وIFN−γ وMIF وIL−4 في الدم. بالإضافة إلى ذلك، وجدت النتائج اختلافًا كبيرًا للغاية في مستويات بروتين TLR2 في المصل بين المرضى الذين لديهم النمط الجيني GA الحامل. اظهرت نتائجنا ان اغلبية عزلات بكتريا المكورات العنقودية الذهبية تنتج أكثر من نوع من المستضدات السطحية المعروفة باسم السموم المحفزة للمناعة، والتي تنشط أعدادًا هائلة من الخلايا الليمفاوية التائية. يرتبط الاليل Gفي جين *TLR2* بزيادة خطر الإصابة بالتهاب اللوزتين المتكرر. يعد هذا الاليل عامل خطر يزيد من فرصة الإصابة بالمرض بالإضافة إلى شدته بسبب ارتباطه الإيجابي بالمرض.كما تعتبر المستويات المرتفعة من TLR2 وIFNγ وMIF وانخفاض مستويات IL−4 جميعها تعتبر علامات تشيرإلى خطورة المرض وموشرات نشخيصية قمهمة

1.Introduction

Tonsils are specialized immune tissues situated near the airways, playing a vital role in the body's defense system by helping to protect against infections and diseases. The tonsils act as a defensive barrier for the body's initial immunological response to infections that have been ingested or inhaled [1-2]. Tonsils include M cells, specialist antigen-capture cells, which make it possible to collect dangerous microorganisms and then initiate the cellular and humeral immune response [3-4-5]. A significant portion of the population had an inflammation of the tonsils brought on by bacterial or viral infection. When recurrent tonsillitis occurs frequently throughout the year, it can negatively impact the patient's quality of life. A variety of different bacteria or viruses can cause tonsillitis, such as influenza virus; β-hemolytic *Streptococcus*, called strep throat; *Staphylococcus aureus* and *Haemophilus*

influenzae [6-8]. Superantigens are dynamic immunostimulatory exotoxins produced by a small number of bacterial pathogens, including S. *aureus*, that all work to activate vast numbers of T cells. Most clinical isolates of Staphylococcus aureus encode more than one superantigen (SAg) gene, and at least 80% of strains carry at least one SAg gene. Some *S. aureus* strains have yielded more than 20 distinct SAgs [9]. When *S. aureus* colonizes the nasal passages, the risk of infection increases fourfold. Furthermore, it is estimated that an endogenous source accounts for around 80% of bloodstream infections caused by *S. aureus,* particularly in patients with impaired immune systems and antibiotic-resistant colonization strains [10]. TLRs, which are pathogen recognition receptors, play an essential role in initiation of the pro-inflammatory immune response to microbial antigens [11]. TLRs have also been shown to have a position in the formation of B-cell and T-cell-mediated responses in addition to instinctive immunity. While there are various types of TLRs, they are all transmembrane proteins having a conserved cytoplasmic domain and an extracellular leucine-rich domain. Lipoproteins are strongly expressed in Gram-positive bacteria outer membrane, have been denominate as the primeTLR2 ligand [12-13]. Recent studies have identified a range of contributing molecules and coreceptors that enhance TLR2 responses by promoting the presentation of microbial components on the cell surface [14-15]. TLR2 heterodimers often start a MyD88-dependent intracellular signaling cascade. This way triggers nuclear factor B translocation to control gene transcription and the following generation of inflammatory cytokines. The gene encoding TLR2 is exited on chromosome 4q32, and over 175 SNPs have been documented for the gene, TLR2 polymorphisms influence patients' susceptibilities to various illnesses, including leprosy, tuberculosis, staphylococcal infections, and even sepsis, are influenced by *TLR2* polymorphisms. Changes in TLR function have been linked to genetic variation of *TLR2*, which encodes one of the most significant TLRs, in different ethnicities worldwide [16]. The aim of present study was designed to inspect the role of rs893629 in *TLR2* gene and its effect on the protein level and certain cytokines in the blood serum, which play a role in the severity of the infection and its development. This was done because bacterial tonsillitis is highly contagious, spreads quickly, and TLR2 is crucial for forming the immune response.

2.Materials and methods

2.1 Study population

This study involved 261 patients of both sexes, along with 89 healthy volunteers, who visited the ENT Unit at Al-Yarmouk Teaching Hospital in Baghdad between November 1, 2022, and January 31, 2023.

2.2 Specimens collections

Each participant underwent a throat swab and blood sample obtained. Five ml disposable plastic syringe was used to puncture the venipuncture incisions of each participant. Each and every blood specimen was spilt into two fractions: 2 ml are placed in an EDTA tube for hematological diagnosis and molecular study, and 3 ml are placed in a gel tube, allowed to form a clot at room temperature and then centrifuged for 15 minutes at a speed of roughly 5000 rpm to get serum. The serum was used to measure the concentrations of TLR2 and other cytokines.

2.3 Isolation and identification of bacterial isolates

The throat swabs were collected and transported to the lab within an hour using a cold box. The swabs were placed on the mannitol salt agar, chromo agar, and blood agar plates. The plates were then incubated for the entire night at 37 ^{o}C. In order to gain a single colony

for further diagnostic processes, the positive culture was streaked [17].After generating a fresh bacterial culture, the Vitek system was employed to identify each isolate.

2.4 Extraction of bacterial DNA and amplification of *SEA, SEB* and *SEC*

Total genomic DNA extraction from all *S. aureus* isolates was performed following the instructions provided in the manual book for the kit (PrestoTM Mini gDNA Bacteria Kit/Geneaid / Korea). The amplification of particular target gene sequences was performed using unique sets of primers designed as detailed in Table 1. This process enabled the detection genes encoding three distinct types of super-antigens in *S. aureus*, which represent the most significant virulence parameters linked to its pathogenicity. Reaction mixture volume: 25 µl total; 5 µl master mix, 3 µl template DNA (50 µg/ml), 1 µl of each primer (50 picamole), and 15 µl distilled water combined. Each of the 35 PCR cycles that were applied to the bacterial DNA consisted of one cycle of initial denaturation, which lasted for three minutes at 95 ºC, 35 cycles of denaturation, which lasted for five minutes at 72 ºC, annealing, which took place for five minutes at 60 ºC for *SEC* and 58 ºC for *SEA* and *SEB*, and one cycle of extension, which lasted for one minute at 72 ºC.

Table 1: Primers for detection *SEA, SEB and SEC* genes in *S. aureus* isolates[9]

Genes		Sequence (5`-3`)	Annealing Temp. (°C)	Product size(bp)
SEA	**F**	**ATTGTTTTGGGGGAGTTTGAAGTT**	**58**	**385**
	R	**TACATTGCGTTTTATTGGTTGCTC**		
SEB	**F**	**GTATGATGATAATCATGTATCAGCAATA**	**58**	**625**
	R	**CGTAAGATAAACTTCAATCTTCACAT**		
SEC	**F**	**GAGTCAACCAGACCCTATGCC**	**60**	**614**
	R	**CGCCTGGTGCAGGCATC**		

Table 2: PCR program for detection of *SEA, SEB* and *SEC* genes in *S. aureus*[9].

Steps		Tm (ºC)	Time (min.)	No. of cycle
Initial Denaturation		**95**	**3**	**1**
Denaturation		**95**	**0.30**	**35**
Annealing	***SEA, SEB***	**58**	**0.30**	
	SEC	**60**		
Extension		**72**	**1**	
Final Extension		**72**	**5**	**1**

2.5 Extraction of human DNA from blood and detection of rs893629 in *TLR2* gene by Tetra arm technique

Genomic DNA can be quickly and easily purified from blood samples using the ReliaPrepTM Blood gDNA Miniprep System. Agarose gel electrophoresis was employed to separate the DNA bands, with a 2% concentration used to verify the size of the PCR products, while genomic DNA bands were found using a concentration of 1.2% [18]. The Tetra ARMS PCR method was used to identify rs893629 in the human *TLR2* gene. Specific primers for the tetra arm technique were designed for this study and provided by Macrogen Inc. (Korea), as shown in Table 2. The lyophilized primer was dissolved in nuclease free water to obtain 100 pmol/µl in the master tube, from which a working solution of 10 pmol/µl was prepared as a working solution. The final volume of the PCR amplification reaction mixture, 25 µL consisted of 5µl Taq PCR Pre Mix 0.5 µl forward inner primer (IF), 0.5 µl inner reverse primer (IR), 0.5 µl forward outer primer(OF), 0.5 µl Outer reverse primer (OR), 1.5 µl template DNA, and 16.5 µl of nuclease-free water. DNA concentration and purity were

accessed at 260/280 absorbance by a nanodrop spectrophotometer. The DNA extraction was stored at −20°C until genotyping.

Table 3: Primer for detection of rs893629 in human *TLR2* Gene

	Size (bp)	Primer	Sequence(5'---3')
SNP rs893629	A allele=188	IF	AGTTCGGGCTTCCATGGATGTAA
	G allel=250	IR	TCTTTAAATTCCACTCCTGATGCGTC
	Two outer primer=389	OF	CTGTCACTGGATTGGGGAAAAAAA
		OR	TTCCAGGTCAGAGAAAAGCGAATC

Table 4: PCR program for detection of rs893629 in *TLR2* gene

Steps	Tm (°C)	Time (min.)	No. of cycle
Initial Denaturation	94	5	1
Denaturation	94	0.5	35
Annealing	60	0.5	
Extension	72	0.5	
Final Extension	72	5	1

2.6 Gel electrophoresis

DNA fragments were separated by agarose gel electrophoresis. After combining with loading dye and running the gel horizontally in 1 X TBE buffer for 1 hour at 5 volts per centimeter, 1.2% agarose was used to verify genomic DNA bands that were isolated from *S. aureus* isolates and from the blood of all study participants. The PCR product was not mixed with a loading buffer because the master mix already contains a density-increasing agent with blue dyes, which serves as a built-in loading dye, allowing the reaction products to be easily visualized during gel electrophoresis. The gel was covered with Tirs Borate EDTA buffer (1X), and the electrophoresis was conducted for two hours at 5 volts per centimeter. The size of the amplified fragment was measured by using a 100 bp DNA ladder as a size marker. To visualize the bands using a gel documentation system, agarose gel was stained with ethidium bromide (0.5µg/ml) for 20-30 min [18]

2.7 Estimation serum level of TLR2, IFN-γ, IL-4 and MIF

The Sandwich ELISA technique was used to estimate serum levels of specific immunological parameters due to its high specificity and sensitivity (Abcam / USA: TLR2 (code: ab227897); INF-γ(code:174443); IL-4(code: ab215089) and MIF (code:100594).

2.8 Statistical analysis

Statistical analysis was performed to test whether group variance was significant or not. Statistical significance was defined as $p \leq 0.05$ or $p \leq 0.01$. Data were expressed as mean ±standard Error and statistical significances were carried out using Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA). Genotypes of *TLR2* were presented as percentage frequencies and significant differences between their distribution in patients and control were assessed by two-tailed Fisher's exact probability. Allele frequencies were calculated by direct gene counting method, while significant departure from Hardy-Weinberg (H-W) equilibrium was estimated using H-W calculator for two alleles.

3. Results

The study included 350 participants divided into two groups: the first group comprised 261 patients (179 females(68.6%)and 82 males (31.4%)) while the second group was made up of 89 healthy participants (38 females, (42.7%) and 51 males (57.3%)). Tonsillitis infections were more common and severe in females than in males. The laboratory culture results of throat swabs from patients revealed that 199(76.2%) samples had positive bacterial culture, 62 (23.8%) samples had negative culture results, and all of the healthy population's throat swabs had negative results. After diagnosis using the vitek system, Gram-positive bacteria were identified as the most common bacterial causative agent of tonsillitis, the results showed that *S. pyogens* represented the most common bacterial species 98(49.2%),69 (34.7%)*S. aureu*s ,32(16.1 %) *H. influenzae*. SAgs which are crucial to the pathogenicity of *S. aureus* isolates were found via PCR utilizing a specially created primer for this investigation. According to the findings all *S. aureus* isolates from tonsillitis patients possesses one or more genes encoded the various types of SAgs, 69 (100%) of the isolates have *SEA*, 57 (82.6%) have *SEB*, and 48 (69.5%) contain *SEC.* Tetra arm technique with designing specific primers used for detection of rs893629 in human *TLR2* gene. The findings revealed that genetic variations in the TLR2 gene are more common in patients with tonsillitis than in healthy individuals, suggesting that these polymorphisms play a significant role in the genetic predisposition to tonsillitis, which is associated with altered TLR2 protein expression, which affect the likelihood of developing disease. Tables (3) show the frequency of genotype distribution and rs893629 alleles for the two groups under investigation. Results showed that severe tonsillitis cases had significantly greater frequencies of the GA genotype and G allele. These results revealed that the occurrence of severe infections was related to the rs893629 in the *TLR2* gene. The AA, GG, and GA genotype frequencies of rs893629 were (17.6% ; 20.6%, and 61.8%) respectively among the patients while 65.2 % for AA, 23.6% for GA, and 11.2% for GG genotypes of healthy control. Sandwich ELISA's high specificity and sensitivity allow it to quantify serum levels of TLR2 and other immunological markers which play an important role in progression of recurrent tonsillitis. Patients' serum TLR2 levels were found to be considerably greater than those of controls, with mean values of (113.27±18.13) pg/ml and (23.40±3.15)pg/ml, respectively as shown table (4). Additionally, the results showed that the pro-inflammatory parameters IFN-γ and MIF were significantly higher in patients than in the healthy controls with mean levels of (54.19±12.4, 84.77±9.39) pg/ml, respectively with significant differences ($P \leq 0.05$). IL-4 is an anti-inflammatory cytokine, is produced by various leukocytes, tissue epithelial cells, and expressed by innate and adaptive immune system cells to perform distinct purpose. Serum of IL-4 serum level was significantly higher in healthy persons (37.95± 1.17pg/ml) in comparison with patients (16.26±0.87pg/ml).

Table 5: Distribution of rs893629 genotypes according to HW equilibrium

Genotypes	Patients (No.=199)		Healthy (No.=89)	
	Observed No. (%)	Expected No.(%)	Observed No.(%)	Expected No.(%)
AA	35 (17.6)	46.8 (23.5)	58 (65.2)	52.0 (58.4)
GA	123(61.8)	99.4 (50.0)	20 (23.6)	32.0(36)
GG	41(20.6)	52.8 (26.5)	11(11.2)	5.0(5.6)
Alleles	No.	%	No.	%
A	193	48.49	136	76.4
G	205	51.51	42	23.6
Total	398	100	178	100

Table 6: Serum level of TLR2 and other cytokines associated with immune pathogenesis pathway of recurrent tonsillitis

Groups	Mean serum level(pg/ml) ± S.E			
	TLR2	IFN-γ	IL-4	MIF
Patients	116.40±18.13	54.19±12.4	16.26±0.87	84.77±9.39
Control	22.70±3.15	15.88±1.92	37.95± 1.17	17.61±1.45
P-value	0.001**	0.001**	0.001**	0.001**

Significant $^{*}P \leq 0.05$, highly Significant $^{**}P < 0.001$, non-Significant $P > 0.05$

Table 7: Association between rs893629 and serum level of TLR2

Genotypes	Mean serum Level of TLR2±S.E ((pg/ml))		P. Value
	Patients (No. 199)	Healthy (No. 89)	
AA	88.38 ± 3.21	19.23±3.15	0.001
GG	93.54 ± 6.54	26.51 ± 0.79	0.001
GA	167.29± 5.52	22.38 ± 2.2	0.001

Significant $^{*}P \leq 0.05$, highly Significant $^{**}P < 0.001$, non-Significant $P > 0.05$

4. Discussion

Studies have shown that females are more prone to repeat bouts of tonsillitis compared to males [19]. Men made up 25% of patients who had tonsillectomy surgery, while women made up 75%. 18 years old was the youngest study participant at the time of surgery, and 62 years old was the oldest [20]. The median age of the study participants was 26 years old, 55.7% of chronic tonsillitis patients were females and 44.3% were males [21]. The majority of the patients (32%) were from age group 1-10 years. Tonsillitis affected 58% of male patients compared to 42% of female patients. Over the years, various theories have been proposed to explain why tonsillitis affects women more frequently than it does men [22]. For instance, women are more likely to contract infections because they spend more time with children who have pharyngitis. For several diseases, sex is an important epidemiological determinant [23]. Oral intercourse can introduce germs into the oropharynx that are not normally present in the flora, which could influence the way tonsillitis appears. Upper respiratory tract infections, including tonsillitis, sinusitis, and otitis, are more prevalent in women. Differently in men and women. The steroid hormone such as estrogens, prolactin, and progesterone-influence the growth and function of both innate and adaptive immunity. Consequently, it is crucial to characterize the hormonal regulation mechanisms of various immune cell types in order to enhance treatment and comprehend the regulatory circuits necessary to maintain a functioning and healthy immune system [24]. Variations in the sample size, the seasons, drugs taken prior to sampling, and the study geographic location could all be contributing factors to the variations in the percentages [25]. Given the correlation between recurrent tonsillitis and tuberculosis, physicians should assess the risks associated with their patients [26]. Age-related declines in pathogen frequency may be due to heightened immunity. Since several organisms populate the oropharynx, Wadeyers ring infections are primarily polymicrobial in nature. *S. aureus* (44.1%) and Group B Streptococcus (35.3%) were the most often isolated bacteria. Two Gram negative bacteria, *Pseudomonas aeruginosa* (2.94%) and *Klebsiella pneumoniae* (8.22%), were also isolated. The complicated illness known as recurrent tonsillitis is influenced by immunological, genetic, and environmental variables. Numerous correlation analyses have suggested that genetic variations in innate immune response genes may be involved as risk factors for recurrent tonsillitis. SAgs increase the likelihood of survival and transmission of organisms

by interfering with normal immune activity. Specifically, tonsillitis has been linked to genetic variants in TLR1, TLR2, TLR4, TLR8, and TLR9(12). This rs893629 increases transcription, changing the serum level of the TLR2 protein. Genetic factors, including changes in gene expression and the presence of specific alleles, contribute to an individual's susceptibility to both acute and chronic tonsillitis, with these genetic variations influencing the development and progression of the disease. Alleles are distinct forms of a gene that have the same genomic location on a chromosome in common [27].*TLR2* gene variant rs893629 is more common in African, Americans, and European linked to a number of illnesses, including thrombosis in SLE [28]. Genetic polymorphisms within the promoter and exons of *TLR2* and *TLR5*were substantially linked to *H. pylori* infection that associated with the development of gastric cancer [29]. In patients with recurrent tonsillitis, the concentration of IFN-γ is three times higher than in healthy individuals, although there is no discernible rise in IL-4 concentrations [30]. Th1-type cytokines IFN-α and IFN-γ are produced at a higher rate than Th2 cytokines IL-6 and IL-4. The quantity of released cytokines in the tonsils and blood, as well as in several types of inflammation, increased in response to TCR and coreceptor activation using an anti CD3 and anti CD28 antibody combination. Tonsils tissue and immune cell were stimulated to produce higher levels of pro-inflammatory cytokines TNF-α, IFN-γ, and IL-2 than anti-inflammatory cytokines IL-4 and IL-10 [31]. Vitamin D exhibits an inversely non-significant association with pro-inflammatory cytokines and a significant positive link with anti-inflammatory cytokines in chronic tonsillitis. In light of this, vitamin D deficiency may influence the frequency of tonsillitis episodes in children by regulating the release of certain cytokines [32]. *TLR* polymorphisms affect immunological dysregulation as well as the emergence of many infections and malignancies. *TLR2* has been linked to over 175 SNPs that raise the risk of tonsillitis, TB, prostate cancer, and thyroid cancer [33]. *TLR2* expression rises in response to inflammatory activation, and these cells become more receptive to TLR2 ligands. This could be a pathogenic mechanism that plays a role in both the inflammatory chronicity of the tonsils and the vascular consequences of sepsis [34]. The presence of primary and/or secondary lesions as sites of pathogen entry may have led to the serum levels of TLR2 in patients being greater than in healthy individuals. There is inflammation, which triggers both innate and adaptive immunological reactions [35]. Because meteorological factors most likely have a major influence on the survival and spread of bacterial and viral pathogens in the environment, they can have an impact on the occurrence of upper respiratory tract infections [36] In the right environment, pathogens can survive longer outside the body, increasing the likelihood that vulnerable individuals would contract the infection. Upper respiratory illness incidence specially tonsillitis is also linked to air pollution in crowded cities. It has been proposed that exposure to air pollution reduces the body's capacity to phagocytose germs, hence increasing the risk of infections caused by these pathogens [37]. This study revealed significant disparities in the common pediatric indications for adenotonsillar surgery, with notable differences observed across age groups, genders, and racial/ethnic populations. According to data, African American patients have a 2.76-fold higher probability than White patients of presenting with upper airway obstruction as the reason for a tonsillectomy [38].

Conclusion

Our investigation found that most *S. aureus* isolates generate a variety of immunostimulatory toxins, commonly referred to as superantigens, which stimulate a significant amount of T cells and have a strong correlation with the immunological response. Carriers of the G allele in the TLR2 gene variant rs893629 have a higher likelihood of developing recurrent tonsillitis, suggesting a potential genetic predisposition to this condition. Positive correlation links the existence of the G allele to a higher probability and severity of

the sickness. High concentrations of TLR2, IFNγ, MIF, and low amounts of IL-4 are significant markers that can potentially reflect the seriousness of the disease.

6.Acknowledgements

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7.Ethical Approval

The protocol of the study was approved by the Ethics Committee of the Iraqi Ministry of Health. Consent was taken from each individual who participated in the study and the personal information of each patient and healthy volunteer was presented in a questionnaire form under the supervision of the consultant and after obtaining approval for obtaining samples from patients and controls. The council of the University of al-Nahrain Institute of College of Biotechnology conducted the study and gave it their approval.

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