*Original Article*

**Microbial Biosystems 10(3) (2025) 2025.354366**
**10.21608/mb.2025.354366.1236**

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

# Evaluation of genetic association between TLR4-D299G polymorphism and tonsillectomy patients infected with *Streptococcus pyogenes*

**Najlaa Abdullah D. AL-Oqaili**

*Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq.*

**ARTICLE INFO**

***Article history***
Received 20 January 2025
Received revised 02 February 2025
Accepted 20 August 2025
Available online 1 September 2025

***Corresponding Editors***
Ghatea, A.
Mustafa, H.

***Keywords***
Tonsillectomy,
S. pyogenes,
Spy1258 gene,
TLR4-D2996G.
rs 4986790(A/G) SNP

**ABSTRACT**

Tonsillitis is currently a global health problem affecting thousands of people whether acute or chronic, for which about 30% undergo surgical intervention (tonsillectomy). The aim of this study is to genetically diagnose *Streptococcus pyogenes* causing tonsillitis and determine the relationship between *TLR4-D299G(A/G) (rs498679)* gene polymorphism in patients with eradicated tonsillitis and healthy individuals. Forty tissue samples were collected from patients of different ages and genders who underwent tonsillectomy at Diwaniyah Teaching Hospital-Iraq. Meanwhile, information was collected from blood samples of the same source from patients who underwent operation for the purpose of extracting *spy1258* gene and 40 blood samples from healthy individuals. The genotype of *SNP TLR4-D299G(A/G)* (*rs4986790*) was determined using T-ARMS-PCR. The results of our current study proved that the main cause of tonsillitis is *S. pyogenes,* with the presence of s*py1258* gene (70%), in relation with the SNP *TLR4- D299G* (*rs4986790*). The homozygous genotype *GG* was (27.5%) for patients and (7.5%) for healthy controls. The genotype AA for patients and healthy group was (52.5%, 75.0%), respectively while genotype *A/G* in patients and healthy group was (20.0%,17.5%) respectively. The current study concludes that heterozygous genotype *A/G* is an insignificant risk factor, which means that patients with the homozygous genotype *GG* were about five times more likely to develop tonsillitis than those with other genotypes.

**Published by Arab Society for Fungal Conservation**

## Introduction

Tonsillitis is a common respiratory disease affecting both children and adults due to bacterial infection, inflammation, and response characteristics associated with the lymphoid tissues of the tonsils. About 9 million new cases of tonsillitis are diagnosed each year (Nabat et al., 2019). Recurrent or specific side effects of tonsil infection or enlargement (dyspnea or dysphagia, mouth breathing, obstructive sleep apnea, etc.) are indicators of tonsillitis, which is perhaps one of the most common human problems. Tonsillectomy is performed in cases of recurrent or persistent side effects of tonsil infection or enlargement. In addition, it is one of the most frequently performed procedures, especially in children, and is the main source of tonsillitis and, moreover, a reservoir for the spread of *Streptococcus pyogenes* (Jeong et al., 2007). It is one of the most frequent Gram- positive microbes in humans, causing a very large number and a wide range of infections (Ralph & Carapetis, 2012).

Since the 1980s, serious infections with *S. pyogenes* have increased worldwide (Amer et al. 2024). *S. pyogenes* is distinguished from other streptococci by its ability to completely lyse red blood cells (so-called beta-lysis) on

* ***Corresponding author Email address***: najlaa67890@gmail.com (**Najlaa Abdullah D. AL-Oqaili**)

sheep blood agar, as opposed to other streptococci, which show mainly partial or no lysis of red blood cells (so-called alpha- or gamma-lysis). This bacterium is responsible for more than 700 million infections and about 517,000 deaths annually worldwide (Armengol et al., 2004). Human defilements range in reality from fairly delicate conditions like pharyngitis to hazardous septicemia and necrotizing fasciitis or myositis. Not only does it cause respiratory infections, but it also causes skin infections, such as impetigo and erythema, as well as rheumatic fever, rheumatic heart disease, and urinary tract infections (Eraso et al., 2020). Antimicrobial immune effectors frequently delay or impede pathogen clearance by avoiding immune recognition. Pathogen-associated molecular patterns (PAMPs) can be regulated by receptors such as Nod-like receptors (NLRs) or Toll-like receptors (TLRs) to prevent their recognition or access. Moreover, toxins and effectors can interfere with signals transmitted through these receptors (LaRock &Nizet, 2015). Upon recognizing these pathogen-associated molecular patterns (PAMPs) and activation of immune response genes, Toll-like receptors (TLRs) recognize membrane-bound patterns (PRRs) that are essential for regulating the human immune system (Creagh & O'Neill, 2006).

Accumulated evidence reveals a role for dysregulated TLR signaling in the pathogenic of infections, autoimmune, allergic, and inflammatory diseases, as well as carcinoma (Corr & O'Neill, 2009; Netea et al., 2012). Many synonymous and nonsynonymous single nucleotide polymorphisms (SNPs) have been identified in the promoter and coding regions of TLR1, TLR2, TLR4, TLR5, TLR7, and TLR9, and their association with infectious and inflammatory diseases (Lee et al., 2012). SNPs in the coding and promoter regions of human TLR4 have been associated with a more incidence of certain infections and inflammatory diseases, such as Gram(-ve) bacterial infections (Awomoyi et al., 2007). Two SNPs have been identified in the TLR4 coding region, encoding an exchange of aspartic acid for glycine and threonine for isoleucine at sited 299 and 399 in extracellular domain, namely D299G and T399I (Lorenz et al., 2002). Recently, carrying D299G TLR4 had been associated with an increased risk of endometritis (Latha et al., 2011), repetitive tonsillitis, tonsillar hypertrophy (Liadaki et al., 2011), and expanded pervasiveness of contaminations in patients with cirrhosis (Guarner-Argente et al., 2010).

The SPY1258 gene encodes a cluster of transcriptional regulators in *S. pyogenes* and thus transcriptional regulators represent a key molecular complex in the adaptation and existence of this pathogenic bacterium (Abraham & Sistla, 2016). Therefore, the present study aimed to genetically characterize *S. pyogenes* causing tonsillitis through the spy1258 gene, and to determine the association between the TLR4 A/G gene polymorphism (rs4986790) and tonsillectomy in patients by ARMS-PCR methods.

## Materials and Methods

### *Tissue sampling*

Forty samples were collected from patients undergoing tonsillectomy at Al-Diwaniyah Teaching Hospital between July 2024 to December 2024 (Iraq). The sample was collected from patients who underwent tonsillectomy for infected tonsils of different ages and sex. The tissue samples were kept in sterile, small tubes at freezing temperature to extract bacterial DNA. At the same time, blood tests were gathered from the same patients who underwent the operation to extract genomic DNA.

### *Bacterial DNA extraction from Tissue Sample*

Bacterial DNA from tonsillitis tissue tests were separated using G-spinTM whole DNA (tissue protocol) according to the company Geneaid, Taiwan. The separation of all DNA was examined by utilizing Nano-drop (Thermo Logical Nano-Drop Light UV Apparent Spectrophotometer, USA). The purity and nature of the DNA (ng/µl) was determined by absorption measurement at 260/280 nm.

### *Blood DNA Extraction*

Geneaid's gSYAN DNA kit extraction kit (frozen blood) contains genomic DNA from blood samples, as directed by the company. The DNA concentration (nanogram/microliter) was measured, as well as its purity, by determining the absorbance at (260/280 nm).

### *Determined genotypic of TLR4-D299G (rs4986790) polymorphism*

The primers in this study; TLR4-D299G A/G rs4986790 gene polymorphism Tetra-ARMS-PCR, were designed using the NCBI-GenBank primer3 database. The Scientific Researcher provided these primers in Iraq, as shown in tables 1 and 2 showing the primers used in the current study.

The mixture was prepared from a 25 µl reaction to amplify TLR4-D299G (A/G) rs4986790 containing 1 µl for each of the forward inner primer (allele A), the reverse inner primer (allele G), and the forward and reverse outer primers,12.5µL G2 green master mix (Promega), 5µL from DNA extracted, 3.5µL nuclease free water. Methods of T-ARMS-PCR was carried out according to (Liadaki et al., 2011).

Then these were placed in PCR thermocycler (BioRad. USA) as follows: The initial denaturation was performed at 95°C for 5 min, and then 35 cycles of denaturation were also performed at 95°C but at 30 s. The next step was annealing at 55°C also for 30 s, and then

extension at 72°C for 30 s. The final extension step was imposed at 72°C for 5 min. The PCR product was then subjected to electrophoresis on a 2% agar gel and UV light imaged under conditions of 100 V and 80 mAcurrent for a full hour.

**Statistical analysis**

The data were carefully analyzed using SPSS version 26, with results presented in clear numbers and percentages. Continuous variables are shown as mean ± standard deviation (SD), while appropriate statistical tests were chosen based on the type of comparison. Specifically, the chi-square test was used to assess relationships between two variables, the t-test was applied to compare means between two groups, and ANOVA was used when comparing means across three or more groups. In addition, the Hardy-Weinberg equation was applied to evaluate the genotype distribution of TLR.

**Results**

***Molecular detection of S. pyogenes from resected tonsil tissue***

In the present study, *S. pyogenes* was identified by analyzing 40 cases of tonsillitis patients who underwent tonsillectomy. The spy1258 gene was found to be able to detect *S. pyogenes* by its specific PCR primer. This study found that 28 (70%) of the tonsillectomy samples had the spy1258 gene as shown in figure (1). Also, the recurrence comparison division of age group and the average age of patients with tonsillitis according to the results of the spy1258 gene PCR is shown in Table (3). The average age was 15.12±3.33 for patients. The current results showed that according to the age groups, there was no significant differences with frequency distribution of patient samples (P=0.068).

***Demographic characteristics of patients with tonsillitis and healthy group***

The present study included 40 patients with tonsillitis who underwent surgical tonsillectomy and 40 healthy controls. Table (4) and figure (2) show the demographic characteristics of the patients and healthy group members. As for age, the mean age of patients with tonsillitis was 14.17 ± 2.97 years and the mean age of the healthy group was 14.31 ± 11.87 years. There was no significant difference between the different groups (P = 0.941). The frequency distribution exhibited no statistically significant difference between patients and healthy group members according to age group (P = 0.772). Regarding gender, overall, 48 (60.0%) males and 32 (40.0%) females were included in the study. In the current study, the patient sample included 26 (65.0%) males and 14 (35.0%) females, while the healthy sample included 22 (55.0%) males and 18 (45.0%) females. As for sex, there was no significant difference in the distribution of frequencies between patients and healthy group (P = 0.519).

***Detection of TLR4-D299G A/G Polymorphism***

The distribution of TLR4-D299G A/G (rs4986790) Polymorphism was detected by T-ARMS- PCR technique. There were three genotypes at this locus; GG, AA, and AG. Only the homozygous wild-type genotype A showed amplification of the allele at a product size of 245bp. The homozygous mutant genotype showed amplification of only the G allele at a product size of 201bp. On the other hand. the heterozygous genotype showed amplification of the A and G alleles at a product size of 245 and 201bp, respectively. Figure (3) showed Tetra -ARMS-PCR product detected healthy internal DNA at 394 base pairs.

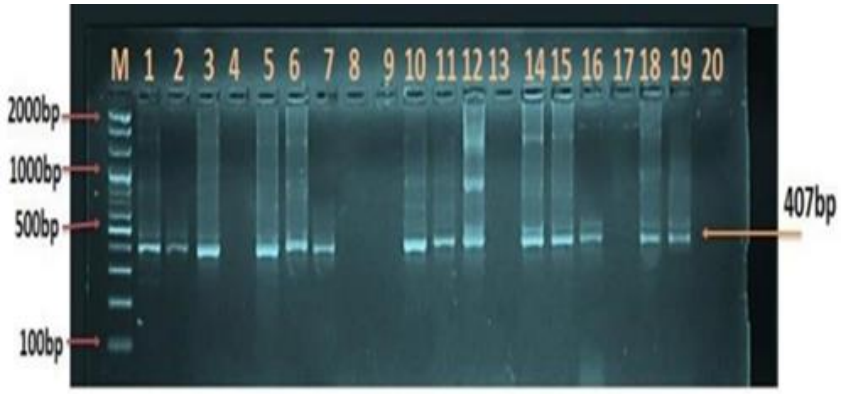


**Fig 1.** PCR products of spy1258 gene detection of *S. pyogenes* from tissue samples of tonsillitis patients by electrophoresis are shown, M (Marker ladder 100-2000bp), the numbers indicate (1-20) some positive *S. pyogenes* isolates at 407bp.

**Table 1** The *S. pyogenes* PCR primer along with product size sequence.

| Primer | Base sequence (5'-3') | Amplification size | Reference |
|---|---|---|---|
| *spy1258* | **F:** AAAGACCGCCTTAACCACCT | 407bp | Abraham & Sistla (2016) |
| | **R:** TGGCAAGGTAAACTTCTAAAGCA | | |

**Table 2** The sequence and amplicon size of the T-ARMS-PCR primers for the TLR4-D299G A/G rs4986790 gene polymorphism.

| Primer | Base sequence (5'-3') | Amplification size |
|---|---|---|
| F- inner primer (G) | CATACTTAGACTACTACCTCGAGGA | 201bp |
| R-inner primer (A) (allele) | TCAAACAATTAAATAAGTCAATAAGAC | 245bp |
| F- outer primer | TAGGCTTCATAAGCTGACTTTA | 394bp |
| R- outer primer | AGTAAGCCTTTTGAGAGATTTG | |

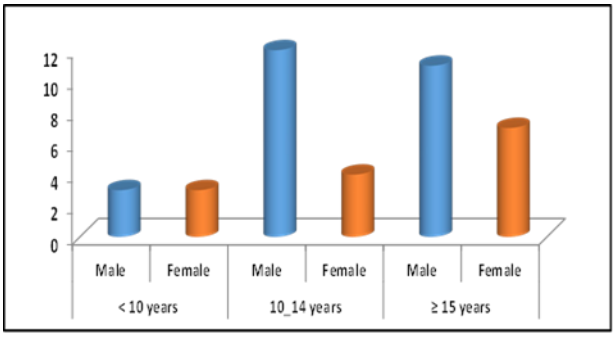


**Fig 2.** Distribution of patients with tonsillitis according to sex and age.

**Table 3** Comparison of the age-specific frequency distribution and mean age of tonsillitis patients based on PCR (*spy1258 gene*) results.

| Characteristic | PCR (spy*1258 gene*) results | | *p* |
|---|---|---|---|
| | ***S. pyogenes*** | **Non-*S. pyogenes*** | |
| **Age (years)** | | | |
| **< 10, *n* (%)** | 2 (7.1 %) | 4 (33.3%) | |
| **10-14, *n* (%)** | 11 (39.3 %) | 5 (41.7%) | |
| **≥ 15, *n* (%)** | 15 (53.6 %) | 3 (25.0 %) | **0.068 ¥ NS** |
| **Total** | 28 (100.0%) | 12 (100.0%) | |
| **Mean ±SD** | 15.12 ± 3.33 | 13.58 ± 1.98 | **0.192** |
| **Range** | **9-19 years** | **9 -17 years** | **† NS** |

NS: not significant at $P > 0.05$; *n*: number of cases; †: independent samples t-test; ¥: Chi-square test; SD: standard deviation.

**Table 4:** *S. pyogenes* infected individuals and control participant were compared about them demographic characteristics.

| Characteristic | Patients | Healthy group | *P* |
|---|---|---|---|
| **Age (years)** | 14.17 ± 2.97 | 14.31 ± 11.87 | **0.941 † NS** |
| **< 10, *n* (%)** | 6 (15.0 %) | 4 (10.0 %) | |
| **10-14, *n* (%)** | 16 (40.0 %) | 18 (45.0 %) | **0.772 NS** |
| **≥ 15, *n* (%)** | 18 (45.0 %) | 18 (45.0 %) | |
| **Sex** | | | |
| **Male** | 26 (65.0%) | 22 (55.0%) | **0.519 ¥ NS** |
| **Female** | **14 (35.0%)** | **18 (45.0%)** | |

NS: no significant at $P > 0.05$; †: independent samples; *n*: number of cases; t-test; ¥: Chi-square test; SD: standard deviation

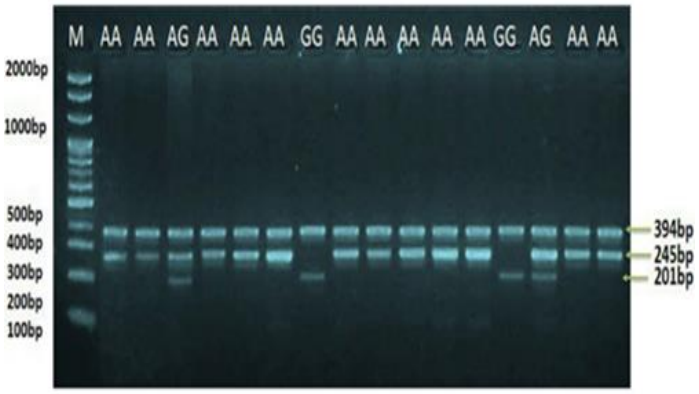


**Fig. 3,** Agarose gel electrophoresis of T-ARMS-PCR products for the TLR4-D299G (A/G) polymorphism. M = DNA marker (2000–100 bp). Lane AA (wild-type) shows the A allele at 245 bp. Lane GG (mutant) shows the G allele at 201 bp. Lane AG (heterozygote) shows both A (245 bp) and G (201 bp) alleles. The 394 bp band represents an internal control.

The distribution of the genotypes AA, AG, and GG of TLR4-D299G A/G (rs4986790) within the healthy group was analyzed using the Hardy Weinberg equation, and the outcomes are presented in table (5). As shown in Table 2, 30 out of 40 healthy individuals had the homozygous wild-type AA genotype, while the homozygous mutant genotype GG was found in 3 out of 40 healthy individuals and the heterozygous genotype AG in 7 out of 40 healthy individuals. According to TLR4-D299G A/G (rs4986790), the genotypes of healthy individuals did not differ significantly from the expected distribution (P = 0.077).

**Table 5** Hardy–Weinberg equilibrium analysis of the genotype distribution. Observed and expected genotype frequencies are compared using the chi-square ($\chi^2$) test. Results show no significant deviation from Hardy–Weinberg equilibrium (NS: non-significant at P > 0.05)

| Genotypes | Observed | Expected | χ2 | *P* |
|---|---|---|---|---|
| Homozygote reference *AA* | 30 | 28.1 | | |
| Heterozygote *AG* | 7 | 10.9 | 5.138 | 0.077 ¥ NS |
| Homozygote variant *GG* | 3 | 1.1 | | |

¥: Chi-square test; NS: Non-significant at *P* > 0.05

Gene analysis including genotypic and allelic, for investigated gene in tonsillitis patients & healthy controls The analysis of allele frequencies and genotypes is related to the TLR4-D299G A/G (rs4986790) SNP. The correlation between tonsillitis control and healthy gatherings is shown in table (6). With respect to mode, there was tremendous distinction in the recurrence conveyance of genotypes between tonsillitis patients and healthy control. The homozygous GG genotype was found to be a not-significant risk factor in risk analysis (OR=5.23). Likewise heterozygous A/G genotype was non- huge gamble factor with an OR of 1.63. This implies that patients with homozygous GG genotype are around five time more at risk to foster tonsillitis sickness in examination with different genotypes. A significant difference was found between patients and control groups (P = 0.002) in allele analysis.

Table 6: TLR4-D299G A/G (rs4986790) polygenotypic frequency tonsillitis patients and healthy groups.

| *TLR4 (rs4986790)* | Tonsillitis patients | Healthy | *P* | OR | 95% CI |
|---|---|---|---|---|---|
| **Genotype frequency** | | | | | |
| *GG* | 27.50% | 7.50% | 0.013* | 5.23 | 1.30-21.9 |
| *A/G* | 8 (20.0%) | 7(17.5%) | 0.202 | 1.63 | 0.51 - 5.19 |
| *AA* | 21(52.5%) | 30(75.0%) | | Reference | |
| **Allele frequency** | | | | | |
| *G* | 30(37.5%) | 13(16.2%) | 0.002* | 3.09 | 1.46-6.52 |
| *A* | 50(62.5%) | 67(83.8%) | | Reference | |

## Discussion

Tonsillitis is a health problem that greatly affects health and standard of living, causing major illnesses and loss of time for school or work. Examining previous studies, the current study is the first to genetically diagnose the Streptococcus bacteria from the tissue of inflamed tonsils that were surgically removed. This study identified the gene 1258 spy in all tissue samples of the removed tonsils. This is in line with (Khalaf et al., 2020) who revealed that all the bacterial isolates of *S. pyogenes* possess the gene 1258spy, but the isolates were collected from laryngitis by swabs. AL-Ameri and AL-Kolaibe (2015) used swabs on children with tonsillitis and laryngitis using the RAPD technique. Our current study contrasts with (Liu et al., 2005) who used these primers (spy1258F and spy1258R) in the Department of Microbiology at the University of Alabama conducting PCR using 60 bacterial strains from human isolates, where only ten of all *S. pyogenes* strains had the spy1258 gene, but did not find 16 non-*S. pyogenes* and 34 other bacteria.

Many techniques for genotyping such as ribonucleotide, electrophoresis, and randomly amplified polymorphic DNA (Fica et al., 2003), have been also used worldwide to genotype *Streptococcus* isolates. The results

of our study disagreed to those of (Cleary et al., 1988), who reported that all *S. pyogenes* isolates carried this gene. Therefore, molecular characterization of the diagnostic gene spy1258 and its association with related genes could offer future insights into species-specific *S. pyogenes* pathogenesis mechanisms, including virulence factors and gene expression. Also, Haukness et al. (2002) established that school-aged children between the ages of 5 and 15 are the primary reservoirs of *S. pyogenes*.

The current study also shows that the incidence of tonsillitis was higher in males than in females. This result is close to what was explained by (Abraham & Sistla, 2016), that males were more affected by tonsillitis by 51% and females by 49%. This result is consistent with a study by (Cavalcanti et al., 2019), who found that males with tonsillitis were 53.7% more likely than females showing 46.3%. Khalaf et al. (2020) also proved that the prevalence rate of *S. pyogenes* (3.3%) of males was found to be higher than that of females (1%). Likewise, Al-Gebori (2007) reported that the prevalence of infection among males was higher than among females in Iraq. Meanwhile, the results of (Fahad, 2018) were consistent indicating thart the percentage of females was more affected by tonsillitis than males. Moreover, the current study was no in line with (AL–Hababy, 2010) who found that females are more susceptible and responsive to infection than males by a ratio of (3:1). The rate of streptococcal infection in this study was (4.3%), which is consistent with (Ahmed et al., 2015), who reported that the prevalence rate of streptococcal pharyngitis was (5.3%). In this study, a group of patients who underwent tonsillectomy due to tonsillitis were analyzed. It was indicated that TLR4 polymorphisms are linked to a higher likelihood of disease recurrence. The *S. pyogenes* a Gram-positive pathogen, causes various infections acute tonsillitis to serious diseases such as recurrent tonsillitis, necrotizing fasciitis, after staphylococcal infection but the causative factors or immune system overreaction remain unclear (Chhatwal & McMillan, 2005).

The distribution of TLR4-D299G A/G SNP (rs4986790) analyzed in this study detected three genotypes; AA, AG, and GG and two alleles; G and A. Risk analysis showed that the heterozygous A/G genotype and homozygous GG genotypic were both not-significant risk factors with an odd ratio. This indicates that patients with the homozygous GG genotype are approximately five times more likely than patients with other genotypes to develop tonsillitis disease. In addition, patients and control groups differed significantly in the allele analysis.

This study is almost identical to (Liadaki et al., 2011) but with different sample size and proportions. TLR4 polymorphism carriers showed a *S. pyogenes* infection risk that is approximately three times greater (for TLR4-D299G, odds ratio equal 2.81, 95 percent CI equal 1.16 to 6.79, P equal 0.038; for TLR4-T3991, OR equal 3.01, 95 percent CI equal 1.29 to 7.02, P equal 0.023) where patients with recurrent tonsillitis had a stronger association. Thus, the presence of TLR4 SNPS is likely to impair signaling, caused by *S. pyogenes* cytolysins, resulting in reduced pathogens or invasive agents and recurrent infections. The study also indicated that the genetic diversity of TLR4 increases the risk of invasive pneumococcal disease a (+ ve) bacterium (Yuan et al., 2008).

Several studies showed an association between TLR4-D299G polymorphism and other infections, one such study was by (Hawn et al., 2009), which revealed that the presence of the SNP TLR4-D299G was related with insurance against intermittent cystitis, a condition caused principally by (-ve) microorganisms. The mechanism by which the TLR4-D299G polymorphism increases the risk of tonsillitis is not fully understood, as our study results showed significant differences in Table 4 between the GG and A/G genotypes. It is thought that the GG genotype may lead to decreased production of inflammatory cytokines, which may make individuals more susceptible to infection.

The complex immune response to pathogens may be influenced by TLR4 mutations in conjunction with other hereditary changes or obtained factors according to previous research for instance, colonization by distinct species (*S. pyogenes* vs. H. influenzae) might be normal, and these species might have developed specific mechanisms to target one another (Kvestad et al., 2005). As a result, the presence of a functional polymorphism may alter immune responses, allowing one species (such as Streptococcus in the tonsils) to colonize more easily while preventing another species (H. influenzae). There are currently no in vitro studies available that could provide additional insight into the precise mechanisms by which the mutant TLR4 receptor actually responds to specific cellular components of *S. pyogenes* and H. influenzae species.

Thus, these differences and discrepancies can be explained by taking into account that the work in previous research does not address all related matters such as the comorbidities of patients with tonsillitis whether or not it was caused by an infection and its genetic relationship with (TLR4-D299G). Thus, the current work confirms that the main cause of the inflamed tonsillitis that was eradicated was *S. pyogenes* bacteria, which was diagnosed by the gene (spy1258) and its relationship with SNP (rs4986790).

In addition, the differences may be due to limited sample size and polygenic effects. This requires further studies with larger sample of patients from different hospitals. Moreover, more parameters need to be studied and compared to obtain more reliable final results and compared them with other studies.

## Conclusions

The current study demonstrated that age and gender do not affect the incidence of tonsillitis. Regarding genotypes, the study demonstrated that the main cause of tonsillitis was S. *pyogenes* through the spy1258 gene, and its relationship with the SNP TLR4-D299G(rs4986790). Hence, the distribution was significantly different from the average genotype frequencies between tonsillitis patients and healthy controls. The homozygous GG genotype was not found to be a significant risk factor according to the risk analysis, whereas the heterozygous A/G genotypic was an insignificant risk factor. This means that patients with the homozygous GG genotype were about five times more likely to develop tonsillitis than those with other genotypes. The study found a significant difference in allele analysis between patient and healthy groups.

## Ethical Approval

The Medical Ethics Committee of Al-Diwaniyah Teaching Hospital in Iraq approved the current work with approval (No. 1106 dated 20/5/2024). The study was conducted in accordance with the moral guidelines taken from the Helsinki Declaration. Furthermore, adult patients and parents of pediatric patients gave verbal and analytical consent before sampling.

## Conflict of Interest

No conflict was reported of researcher interests.

## Acknowledgements

The authors express gratitude to the Faculty of Biotechnology, including the Molecular Biology Laboratories, for their assistance. We should not forget the teaching hospital, its medical staff and patients, from whom tonsillectomy and blood samples were taken after the patients' consent. I would like to thank and respect the referees for their sound understanding of the article and their insightful scientific comments.

## Funding

No financial amount was allocated from any organization for the current work.

## References

Abraham T, Sistla S. (2016). Identification of *Streptococcus pyogenes*–Phenotypic Tests vs Molecular Assay (spy1258PCR): A Comparative Study. Journal of clinical and diagnostic research: JCDR,10(7),DC01. https://doi.org/10.7860/JCDR/2016/20053.8093

Ahmed Y T, Zbar N S, Al-Shaibani A B. (2015). Detection of Hyaluronidase and Streptokinase Virulence Factors from *Streptococcus pyogenes*. Al-Nahrain Journal of Science, 18(1), 136-140. https://doi.org/10.22401/JNUS.18.1.20

AL-Ameri G A, AL-Kolaibe A M. (2015). Identification and genotypic analysis of *Streptococcus pyogenes* isolated from pharyngitis and tonsillitis infected children in IBB city in Yemen. Journal of Microbiology and Antimicrobials, 7(3), 21-27. https://doi.org/10.5897/JMA2013.0277

Al-Gebori A. (2007). Bacterial study of beta–hemolytic streptococci causing tonsillopharyngitis and correlation of its MIC and MBC with hemolysin production M. Sc. Thesis, College of Medicine, Baghdad University, Iraq].

AL–Hababy H. (2010). Bacteriological and immunological study on group A *Streptococcus pyogenes* isolated from patients in babylon province MSC Thesis. College of Medicine. University of Babylon.

Amer S, Allam N, El-shanshory M, Ibrahim F, El-shafey A. (2024). Laboratory diagnosis and treatment of pathogenic bacteria isolated from children with cervical lymphadenopathy at Tanta University Hospital. Microbial Biosystems, 9(1), 63-75. doi: 10.21608/mb.2024.283357.1095

Armengol C E, Schlager T A, Hendley J O. (2004). Sensitivity of a rapid antigen detection test for group A streptococci in a private pediatric office setting: answering the Red Book's request for validation. Pediatrics, 113(4), 924-926. https://doi.org/10.1542/peds.113.4.924

Awomoyi A A, Rallabhandi P, Pollin T I, Lorenz E, Sztein M B, Boukhvalova M S, Hemming V G, Blanco J C, Vogel S N. (2007). Association of TLR4 polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children. The Journal of Immunology, 179(5), 3171-3177. https://doi.org/10.4049/jimmunol.179.5.3171

Cavalcanti V P, Camargo L A d, Moura F S, Melo Fernandes E J d, Lamaro-Cardoso J, Braga C A d S B, André M C P. (2019). *Staphylococcus aureus* in tonsils of patients with recurrent tonsillitis: prevalence, susceptibility profile, and genotypic characterization. Brazilian Journal of Infectious Diseases, 23, 8-14. https://doi.org/10.1016/j.bjid.2018.12.003

Chhatwal G S, McMillan D J. (2005). Uncovering the mysteries of invasive streptococcal diseases. Trends in Molecular Medicine, 11(4), 152-155. https://doi.org/10.1016/j.molmed.2005.02.001

Cleary P P, Kaplan E, Livdahl C, Skjold S. (1988). DNA fingerprints of *Streptococcus pyogenes* are M type specific. Journal of infectious diseases, 158(6), 1317-1323. https://doi.org/10.1093/infdis/158.6.1317

Corr S C, O'Neill L A. (2009). Genetic variation in Toll-like receptor signalling and the risk of inflammatory and immune diseases. Journal of innate immunity, 1(4), 350-357. https://doi.org/10.1159/000200774

Creagh E M, O'Neill L A. (2006). TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. Trends in immunology, 27(8), 352-357. https://doi.org/10.1016/j.it.2006.06.003

Eraso, J. M., Kachroo P, Olsen R J, Beres S B, Zhu L, Badu T, Shannon S, Cantu C C, Saavedra M O, Kubiak S L. (2020). Genetic heterogeneity of the Spy1336/R28—Spy1337 virulence axis in *Streptococcus pyogenes* and effect on gene transcript levels and pathogenesis. PLoS One, 15(3), e0229064. https://doi.org/10.1371/journal.pone.0229064

Fahad H M. (2018). Types of Aerobic Bacteria Isolated from Iraqi Patients with Acute Tonsillitis and their Susceptibility to Different Antibiotics. J. Pure Appl. Microbiol, 12(4), 1855-1859. https://doi.org/10.22207/JPAM.12.4.20

Fica A, Fernández J, Ebensperger G, Cona E, Galanti A, Alonso C, Ulloa M T, Frola A M, Prat S. (2003). Molecular epidemiology of a *Streptococcus pyogenes* related nosocomial outbreak in a burn unit. Revista Medica de Chile, 131(2), 145-154. https://doi.org/10.4067/S0034-98872003000200003

Guarner-Argente C, Sánchez E, Vidal S, Román E, Concepcipolón M, Poca M, Sánchez D, Juárez C, Soriano G, Guarner C. (2010). Toll-like receptor 4 D299G polymorphism and the incidence of infections in cirrhotic patients. Alimentary pharmacology & therapeutics, 31(11), 1192-1199. https://doi.org/10.1111/j.1365-2036.2010.04291.x

Haukness H A, Tanz R R, Thomson Jr R B, Pierry D K, Kaplan E L, Beall B, Johnson D, Hoe N P, Musser J M, Shulman S T. (2002). The heterogeneity of endemic community pediatric group a streptococcal pharyngeal isolate and their relationship to invasive isolates. The Journal of infectious diseases, 185(7), 915-920.https://doi.org/10.1086/339407

Hawn, T R, Scholes D, Li S S, Wang H, Yang Y, Roberts P L, Stapleton A E, Janer M, Aderem A, Stamm W E. (2009). Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women. PLoS One, 4(6), e5990. https://doi.org/10.1371/journal.pone.0005990

Jeong J H, Lee D W, Ryu R A, Lee Y S, Lee S H, Kang J O, Tae K. (2007). Bacteriologic comparison of tonsil core in recurrent tonsillitis and tonsillar hypertrophy. The Laryngoscope, 117(12), 2146-2151. https://doi.org/10.1097/MLG.0b013e31814543c8

Khalaf N Z, Najeeb L M, Abdul-Jalil A A. (2020). Molecular study of spy1258 gene in *Streptococcus pyogenes* isolated from pharyngitis patients in Fallujah city. Medico Legal Update, 20(1), 585-590. https://doi.org/10.37506/mlu.v20i1.424

Kvestad E, Kværner K J, Røysamb E, Tambs K, Harris J R, Magnus P. (2005). Heritability of recurrent tonsillitis. Archives of Otolaryngology–Head & Neck Surgery, 131(5), 383- 387. https://doi.org/10.1001/archotol.131.5.383

LaRock C N, Nizet V. (2015). Inflammasome/IL-1β responses to streptococcal pathogens. Frontiers in Immunology, 6, 518. https://doi.org/10.3389/fimmu.2015.00518

Latha M, Vaidya S, Movva S, Chava S, Govindan S, Govatati S, Banoori M, Hasan Q, Kodati V L. (2011). Molecular pathogenesis of endometriosis; Toll-like receptor-4 A896G (D299G) polymorphism: a novel explanation. Genetic Testing and Molecular Biomarkers, 15(3), 181-184. https://doi.org/10.1089/gtmb.2010.0178

Lee Y H, Lee H S, Choi S J, Ji J D, Song G G. (2012). Associations between TLR polymorphisms and systemic lupus erythematosus: a systematic review and meta-analysis. Clin Exp Rheumatol, 30(2), 262-265.

Liadaki K, Petinaki E, Skoulakis C, Tsirevelou P, Klapsa D, Germenis A E, Speletas M. (2011). Toll-like receptor 4 gene (TLR4), but not TLR2, polymorphisms modify the risk of tonsillar disease due to *Streptococcus pyogenes* and *Haemophilus influenzae*. Clinical and Vaccine Immunology, 18(2), 217-222. https://doi.org/10.1128/CVI.00460-10

Liu D, Hollingshead S, Swiatlo E, Lawrence M L, Austin F W. (2005). Rapid identification of *Streptococcus pyogenes* with PCR primers from a putative transcriptional regulator gene. Research in microbiology, 156(4), 564-567. https://doi.org/10.1016/j.resmic.2005.01.010

Lorenz E, Mira J P, Frees K L, Schwartz D A. (2002). Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. Archives of internal medicine, 162(9), 1028-1032. https://doi.org/10.1001/archinte.162.9.1028

Muhtarova A, Mihova K, Markovska R, Mitov I, Kaneva R, Gergova R. (2020). Molecular emm typing of Bulgarian macrolide-resistant *Streptococcus pyogenes* isolates. Acta Microbiologica et Immunologica Hungarica,67(1),14-17. https://doi.org/10.1556/030.66.2019.033

Nabat Z N, ALateef B A, Hussain I M. (2019). Bacteriological and immunological study of patients with tonsillitis in Hila City. Iraqi journal of biotechnology, 18(2).

Netea M G, Wijmenga C, O'neill L A. (2012). Genetic variation in Toll-like receptors and disease susceptibility. Nature immunology, 13(6),535-542. https://doi.org/10.1038/ni.2284

Ralph A P, Carapetis J R. (2012). Group a streptococcal disease and their global burden. Host- pathogen interactions in streptococcal diseases,1-27. https://doi.org/10.1007/82_2012_280

Yuan F F, Marks K, Wong M, Watson S, De Leon E, McIntyre P B, Sullivan J S. (2008). Clinical relevance of TLR2, TLR4, CD14 and FcγRIIA gene polymorphisms in *Streptococcus pneumoniae* infection. Immunology and cell biology, 86(3), 268-270. https://doi.org/10.1038/sj.icb.7100155