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Genetic Relationship Between Chronic Otitis Media Infection And Tlr4 Gene In Al-Qadisyah Province / Iraq

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Abstract: *Otitis media is considered the more diseases commonly spread related to hearing loss , This study is designed to focus on TLR4 expression that stimulates the result presence of LPS in otitis media .To detect the genotype of more infection in otitis media on blood patient's sample expression in PCR Technical.*

In the current study blood specimens we get of 50 patients and chronic Otitis media and 50 blood samples from healthy people to Extract and dissection of the DNA blood gram-negative bacteria and evaluate the mechanical work of LPS and its effect on Toll like receptor4.

The results show most patients with P. arogenosia isolates have GG genotype 9 in comparison with another genotype AG, 15 and AA 26 and the control group show GG genotype 4 in comparison with other genotype AG 11, and AA 36 .

The study is showed a high rate of chronic otitis media in patients with the GG genotype, suggesting that this genotype may be a risk factor compared to the control group. On the other hand, the AA genotype was found to be low in patients but high in the control group, indicating a possible is protected factor.

Keywords: Toll-like receptors (TLRs), tumor necrosis factor-alpha (TNFα) , lipopolysaccharide (LPS), chr0nic 0titis media (COM)

1.Introduction

The formation of the ear begins in the 42 day of pregnancy through fetal development [1]. The ear in the attack is found to be in three areas : the outer ear, the middle ear, and the inner ear [2]. middle ear has an unregulated form and is line up with mucous membrane [3] The ear is responsible for both hearing and equilibrium [4].

In children, the Eustachian tubes are be short and high horizontal due to the smaller size of the skull, which makes them less efficiency at equalizing pressure. As the head grows and the skull expands, the Eustachian tubes become high columnar, improving the natural pressure balance in the middle ear [5]. Otitis media (OM) is an infectious defect that is a common cause of hearing loss in all ages. Both acute and chronic OM contribute to significant healthcare utilization, including antibiotic prescriptions and surgical procedures[6].

Understanding the tasks of OM-attachment genes may availability opportunities to diagnose, treat or prevent. Genetic and molecular studies have revealed associations between OM and various mechanisms, including the development of the cavity in middle ears and Eustachian tube, immune response, bacterial attachment and viral infection, regulation of the extracellular matrix, and middle ear clearance [7].

Toll-like receptors (TLRs) play a crucial role in stimulating the innate immune response, and their dysregulation has been implicated in the pathogenesis of chronic otitis media (COM). TLR4 polymorphisms have been associated with susceptibility to acute otitis media and chronic otitis media with effusion [8)]. Studies have shown that TLR4 is not expressed in normall middle ear samples but is readily detected in COM [9]. Gram-negative bacterial infections, including those caused by lipopolysaccharide (LPS), have been used to establish CSOM models as LPS can be recognized by TLR4 to trigger proinflammatory reactions [10].

The TLR4 risk haplotype was found to exhibit reduced TNFα protein production in myeloid dendritic cells in response to LPS stimulation compared to non-risk haplotypes. When evaluating TNFα cytokine mRNA production after LPS stimulation in PBMCs, carriers. TNFα has multiple transcription start sites, including an antisense transcription start site located 3' of the gene [11]. For functional studies on TLR4 signaling, we recruited ten age- and sex-matched pairs with either risk or protective TLR4 haplotypes. Peripheral blood was collected from each pair on the same day and analyzed in parallel. Mono nuclear cells were isolated for mRNA analysis. Given the complexity of mRNA studies on TLR4, we opted to use the downstream target TNFα as a proxy for the expression of biologically functional TLR4 protein [12]. Inflammatory and immunological responses of endothelial ear cells stimulated with LPS have been extensively investigated. LPS indirectly activates endothelial cells through inflammatory mediators released by macrophages and immune cells, such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and interferons (IFNs) [13]. Increased TLR4 expression in monocytes was discovered to be involved in the aetiology of COM, as evidenced by the finding that monocytes express TLR4 at a higher level in lymphocytes (14).

2.Methodology.

The present study aimed to isolate and identify bacteria responsible for causing inflammation in otitis media. Samples were collected from different patients, encompassing various age groups and genders, in Al-Diwaniya General Teaching Hospital and Al_Diwaniya Maternity and Pediatrics Teaching Hospital, located in the city of Al-Dewaniyah. The data collection period spanned from October 20, 2022, to January 28, 2023 .2 cc of blood was collected in an EDTA tube, and relevant information about the patient was recorded**.** blood samples from heallthy individuals were collected in EDTA tubes for genetic studies. All blood samples were stored in a refrigerator at the hospital for a specific duration.

Genomic DNA Extraction.

Using the Gsyan DNA Extraction Kit (Frozen Blood) from Geneaid, USA, and adhering to the manufacturers' instructions, genomic DNA was extracted from blood specimens.

Tetra- ARMS-PCR Method

Patients with Pseudomonas aeruginosa infections and specimens from the healthy group were identified and genotype for the TLR4 RS5030717- 55 A/G gene polymorphic Utilize G T-ARMS-PCR.

Data Analysis

The collects data from the study underwent rigorous tests to specify and find out the effectiveness of the treatments and determine the significance of the observed

differences among the groups. Descriptive statistics were employed to summarize the data, including measures such as mean, standard deviation, and frequency distributions. These summary statistics provided an overview of the central tendency and variability of the measured parameters.\ To compare the treatment groups, inferential statistical tests were performed. Analysis of Variance (ANOVA) was used to assess the overall differences among the groups for continuous variables, such as white blood cell count, lymphocyte levels, red blood cell count, and IFN-gamma levels. Post-hoc tests, such as Tukey's test, were conducted to determine specific pairwise comparisons between the treatment groups. The significance level was set at $p < 0.05$ to determine statistical significance. Statistical software, such as SPSS or R, was utilised for data analysis, providing appropriate outputs, including p-values, confidence intervals, and effect sizes. Chapter Three Materials and Methods 58 Additionally, graphical representations, such as bar charts, line graphs, and box plots, were created to visually illustrate the differences and trends in the data. The findings of the data analysis provided insights into the efficacy of the A. bisporus extract and its potential impact on A. fumigatus infections. The statistics analysis facilitated the identification of significant treatment effects and supported the conclusions drawn from the study**.**

3.Results and Discussion.

The present study included a total of 100 participants, consisting of 50 patients diagnosed with otitis media infection and 50 apparently healthy individuals as control subjects. These studies provide evidence for the involvement of TLR (Toll like receptor) gene polymorphisms and variations in the susceptibility to chronic otitis media and otitis media with effusion. Specifically, TLR4 polymorphism was found to be more frequent in patients with chronic otitis media.

The study also investigated the relationship between different mucosal changes in CSOM without cholesteatoma and TLR4 polymorphisms. However, no statistically significant differences were observed in the genotype distribution of both TLR4 polymorphisms among patients with different mucosal changes in COM.

Overall, the study suggests that genetic variations in TLR2 and TLR4 genes may play a role in determining susceptibility to chronic otitis media. However, more research is needed to further understand the specific impact of these genetic variations on the development and progression of different forms of otitis media..

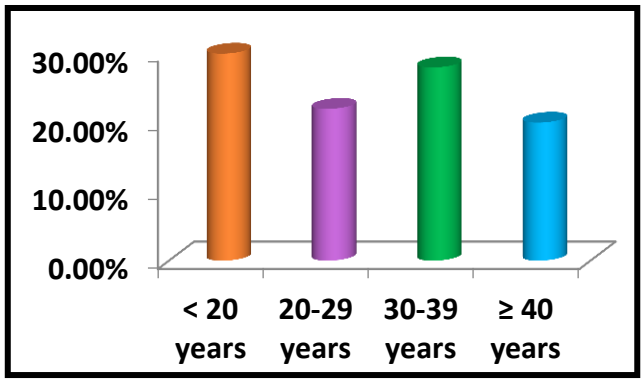

Figure (1) : Distribution of patients with otitis media infection according age group.

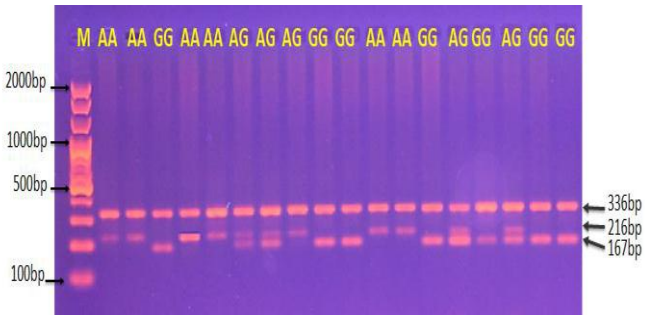

Figure (2): Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of for TLR4 rs5030717-A/G gene polymorphism. Where M: marker (3000-100bp). The lane (AA) wild-type homozygote showed only an A allele at 216bp T-ARMS-PCR product. The lane (GG) mutant type homozygote showed only the G allele at 167bp T-ARMS-PCR product, whereas the (AG) heterozygote was showed as both A and G alleles at 216bp and

167bp T-ARMS-PCR product. The outer internal control was observed at 326bp T-ARMS-PCR product.

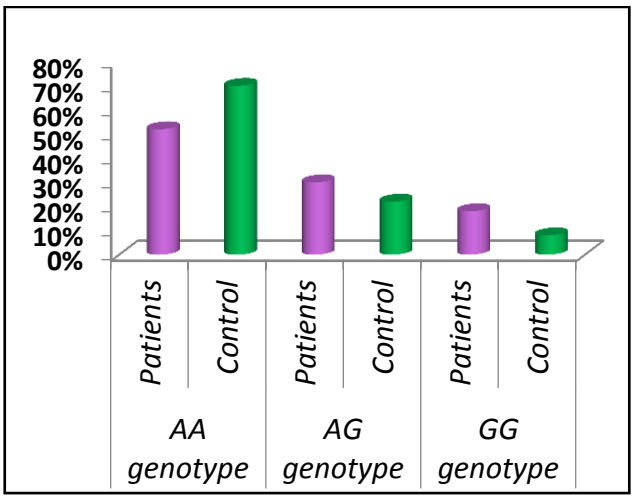

Figure (3) : Distribution of patients with otitis media infection and control subject according to genotype.

Table (1): Demographic Characteristics of patients with otitis media infection and control subjects.

Characteristic	Patients *n* = 50	Control *n* =50	*P*
Age (years)			
Mean ±SD	27.76 ±14.85	27.87 ±9.99	0.963 † NS
Range	4-61	4 -57	
< 20, *n* %)	15 (30.0 %)	12 (24.0 %)	0.138 ¥ NS
20-29, *n* (%)	11 (22.0 %)	20 (40.0 %)	
30-39, *n* (%)	14 (28.0 %)	14 (28.0)	
≥ 40, *n* (%)	10 (20.0 %)	4 (8.0 %)	
Gender			
Male, *n* (%)	33 (66.0%)	31 (62.0%)	0.677 ¥ NS
Female, *n* (%)	17 (34.0%)	19 (38.0%)	

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

When The comparison of genotypes and allele frequencies concerning *TLR4 (rs5030717)* SNP between patients and healthy control was shown in GG in patients 9 (18.0%) and healthy group and 4 (8.0 %) . the GG mutant type homozygote contains on only G allele representing risk factor . where all patients with the mutant genotype (*GG genotype*) have *P. aeruginosa* bacterial infection. A/G(heterozygote allel)is contain on both A and G allele at in patients 15 (30.0%) and healthy group 11 (22.0 %) . (AA) wild type homozygote is contained on only A allele in patients 26 (52.0 %) and healthy group 35 (70.0 %) in Al-Dewaniya city. In a study conducted by [15,16], it was found that a single nucleotide polymorphism (SNP) called rs5030717 (A/G) located in the third intron of the TLR4 gene was significantly associated with an increased risk of otitis media (OM). The minor allele G of this SNP was identified as a risk factor, with an odds ratio (OR) of 1.33 and a p-value of 0.003.[17]. Additionally, TLR4 haplotypes were found to be associated with an increased risk of OM. 100 Further investigations were conducted to explore the functional characteristics of different TLR4 haplotypes in peripheral blood. It was observed that individuals carrying the TLR4 risk haplotype exhibited lower expression of Tumor necrosis factor alpha (TNFα) protein in myeloid dendritic cells upon stimulation with the TLR4 ligand lipopolysaccharide (LPS). Changes in TNFα mRNA levels were also detected in peripheral blood mononuclear cells (PBMC) after LPS stimulation. These findings suggest that the TLR4 risk haplotype may affect immune responses and inflammatory processes associated with OM[18,19].

The results indicate that there was in the frequency distribution of TLR4 (rs5030717) genotypes among patients with otitis media infection, as determined by the results of PCR targeting the 16S rRNA gene (16SrRNA) (P = 0.727). Among patients with *Pseudomonas aeruginosa* isolates, the majority had the GG genotype, with 9 individuals (45.0%) having this genotype. The AA and AG genotypes were observed in 4 (20.0%) and 7 (35.0%) patients, respectively.

Table (2): Comparison of the frequency distribution of *TLR4 (rs5030717) genotypes* of patients with otitis media infection according to the results of PCR (*16SrRNA*).

Characteristic	Patients with *P. arogenosia* isolate *n* = 20	*P*
TLR4 (rs5030717)		
AA, *n* (%)	4 (20.0%)	**0.387 ¥ NS**
AG, *n* (%)	7 (35.0%)	
GG, n (%)	9 (45.0%)	

S¥: chi-square test; **D**: standard deviation; ***n***: number of cases; **S**: significant at $p > 0.05$**.**

4.Conclusions

The study identified and characterized bacterial infections causing otitis media, with a significant presence of Pseudomonas aeruginosa, K. pneumonia, P. mirabilis, and E. coli in otitis media cases. The TLR4 rs5030717-A/G gene polymorphism was associated with Pseudomonas aeruginosa infection in otitis media patients.The T-ARMS-PCR technique proved effective in detecting and genotyping the TLR4 rs5030717-A/G gene polymorphism. Agarose gel electrophoresis was successfully utilized for analyzing the T-ARMS-PCR products.

Ethical approval

The results was matching with to the College of Medical Biotechnology at Al Qadisiyah University information's.

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