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Impact of Toll-like receptor 2 (rs5743708) gene polymorphism in pediatric pneumonia: risk and severity

Samar M. Abd El-Hamid[1], Alshaymaa A. Abd Elalim[1], Eatemad N. A. Mansour[2], Shimaa Moustafa[2], Eman M. Moazen[3], Walaa H. Abdo[4], Amal K. A. Abou Elnour[4] and Asmaa A. Elsheikh[5]

[1]Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[2]Department of Pediatrics, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[3]Department of Chest disease, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[4]Department of Radio-diagnosis, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[5]Department of Community & Occupational Medicine, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

Corresponding author: Samar M. Abd El-Hamid, Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.
Email: drsamar33@yahoo.com.

Abstract

Pediatric pneumonia is a common respiratory infection that affects children and is thought to be a major source of mortality and morbidity worldwide, particularly in low- and middle-income nations. Toll-like receptor2 (TLR2) is an important receptor involved in the recognition of bacterial pathogens and the activation of the immune response. Genetic variability in TLR2 may partially explain individual differences in susceptibility to infections. The purpose of this study was to investigate the possible contribution of the TLR2 (rs5743708) variant to the risk and severity of pediatric pneumonia infection. The study included 100 pediatric patients diagnosed with pneumonia and 100 normal controls who were age and gender matched. Real-time polymerase chain reaction (PCR) was used to genotype participants for the TLR2 (rs5743708) variant. The analysis revealed that children with the TLR2 (rs5743708) (G/A) genotype showed a 2.52-fold greater risk of having pneumonia (OR: 2.52; 95% CI: 1.32-4.79; p = 0.005) in comparison with patients who have wild homozygous genotypes. Furthermore, we observed that the TLR2 (rs5743708) (A) allele is connected to a greater risk of pneumonia infection in children (OR: 1.612; 95% CI: 1.07-2.43; p = 0.023) but did not significantly influence infection severity. In conclusion, children with the TLR2 (rs5743708) mutant (G/A) genotype and (A) allele had a significantly higher risk of having pneumonia, but they were not at high risk for the severity of the infection.

Keywords: Pneumonia, Toll-like receptors2, Pediatric Respiratory Severity Score, polymorphism.

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Introduction

Pediatric pneumonia is one of the most common infectious diseases and is related to a significant mortality and morbidity rate around the world. Around 152 million young children worldwide have clinical pneumonia each year, predominantly in low- and middle-income

nations. It was estimated that 8.7% of them required hospitalization due to severe pneumonia.[1] As per the 2014 Egypt Demographic and Health Survey, children under the age of five make up about 13.4% of the overall population.[2] According to official estimates, there were between 0.11 and 0.20 episodes of pneumonia per child in Egypt per year and constituted 19% of under-five mortality.[3]

Different host factors, including the effectiveness of immune responses to infection, which require the recognition of pathogen-associated molecular patterns (PAMPs) to differentiate self from infectious agents, as well as pathogen discrimination, may play a role in the variability in susceptibility to pneumonia.[4] It is believed that the pathogen recognition system's genetic diversity contributes to individual differences in susceptibility to infections.[5]

Toll-like receptors (TLRs) are a kind of pattern recognition receptor (PRR) that recognizes viral, bacterial, or fungal structures of molecules or nucleic acids and causes systemic inflammation. TLR activation plays a crucial role not only for detecting different PAMPs but also for activating and directing the adaptive immune system via regulating the antigen-presenting cells' costimulatory molecules.[6]

Among the various TLRs, toll-like receptor 2 (TLR2) is important in antibacterial and antiviral immunity, recognizing lipoproteins and peptidoglycans from both Gram-positive and Gram-negative bacteria, as well as lipopolysaccharides (LPS) and lipoteichoic acid from Gram-positive bacteria.[7]

The TLR2 (rs5743708) variant represents a missense mutation that impacts the intracellular region of the TLR2 protein's structure and results in the generation of a nonfunctional protein because of the substitution of the amino acid arginine for glycine at position 753 of the protein (Arg753Gln) and is linked to the incidence and development of various diseases.[8-14] This study evaluated the possible contribution of genetic variations in the TLR2 (rs5743708) variant on the risk and severity of the development of pediatric pneumonia infection.

Subjects and Methods

This was a case-control study, performed at Al-Zahraa University Hospital, Cairo, Egypt, during the period between January 2023 and July 2023. A total of 200 participants, aged between one month to 15 years, were selected from the Department of Pediatrics at Al-Zahraa University Hospital. The participants were split into two groups: Group I, included 100 children, diagnosed with pneumonia, and Group II, included 100 apparently healthy controls of matched age and gender. Group I was further subdivided into two categories: Subgroup I A (n=50), non-severe (mild/moderate) pneumonia patients and Subgroup I B (n = 50), patients who have severe pneumonia.

Pneumonia diagnosis and severity were assessed at hospital admission using the revised World Health Organization (WHO) recommendations for 2014 (for children aged 2 months to 5 years)[15] and the Pediatric Respiratory Severity Score (PRESS) (for children aged 5 to 15 years).[16] The diagnosis was confirmed based on chest radiograph.

Patients with less than one month or > 15 years old, suffering from cough that lasted more than 14 days; being suspected of having tuberculosis or an underlying chronic respiratory illness or persistent asthma were excluded from the study. Also, severely malnourished children having neurological illnesses or immunodeficiency disease were excluded from the study. Similarly, patients with no radiological evidence of consolidation were excluded as well.

Participants underwent the following: complete clinical data including age, gender, disease duration. Patients' data for blood cultures, vital signs, and oxygen saturation, radiological examination by chest X-ray; posterior-anterior erect view or anterior-posterior view were obtained from patients' hospital records.

Blood samples were collected from all study participants and used for the following

investigations. Routine laboratory testing, such as a complete blood count (CBC), was performed using a fully automated hematology analyzer (CELL-DYN Ruby, Abbot, USA), according to the manufacturer's instructions. The modified Westergren method was used to determine the erythrocyte sedimentation rate (ESR).

C-reactive protein (CRP), liver, and kidney function tests were performed using a fully automated chemical analyzer (Cobas C 311, Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Serum levels of interleukin-6 (IL-6) was performed using an immunoassay analyzer (Cobas e411 system, Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions.

Blood culture for study participants was done using a blood culture system (Ref. No. 410853, BACT/ALERT® 3D 60; BioMérieux, USA). Culture bottles (BACT/ALERT® PF Plus from BioMérieux, USA) were used with BACT/ALERT 3D and VIRTUO® automated detection system in procedures for the qualitative detection of aerobic and facultative anaerobic microorganisms from blood in pediatric patients.

Finally, genotyping of the TLR2 (rs5743708) variant was performed for study subjects using a real-time polymerase chain reaction (PCR) assay. For this, genomic DNA was extracted using commercial kits (Lot. No. 01167511, GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Scientific, USA), according to the manufacturer's instructions. The purity and concentration of the prepared DNA samples were examined using a UV/VIS spectrophotometer (QIAxpert, QIAGEN, Hilden, Germany) before being stored at −20°C.

Genotyping of TLR2 (rs5743708) by Real-time PCR using TaqMan® SNP Genotyping Assay

TaqMan® SNP genotyping Assay of TLR2 rs5743708 was used (Lot. No. P211222-005A10, Applied Biosystems, Thermo Fisher Scientific, USA). The assay contained sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest, two TaqMan® minor groove binder (MGB) probes with non-fluorescent quenchers (NFQ): one VIC™-labelled probe to detect the allele 1 sequence and one FAM™-labelled probe to detect allele 2 sequence, the context sequence was (ATTCCCCAGCGCTTCTGCAAGCTGC[A/G] GAAGATAATGAACACCAAGACCTAC).

The genotyping PCR reaction was conducted in 100 µl PCR tubes. Each reaction total volume was 20 µl, contained 10 µl of TaqMan Genotyping Master Mix (Lot. No. 01176979), 0.5 µl of TaqMan® SNP Genotyping Assay, and 9.5 µl of diluted DNA template (5.5 µl nuclease free water, Lot. No. 01069419, and 4 µl template).

The PCR process consisted of 25 to 50 cycles, each of an initial denaturation at 95°C to separate the nucleic acid double chain, annealing at 58°C for binding of primers, and extension by DNA polymerase at 72°C. Data were collected and read based on fluorescence signals using a Rotor Gene real-time system (QIAGEN, Hilden, Germany). The allelic discrimination data were plotted as a comparison of allele 1 (VIC™ dye) and allele 2 (FAM™ dye) using real-time PCR instrument software (QIAGEN, Hilden, Germany).

Statistical Analysis

The statistical package for social sciences (SPSS Inc., Chicago, Illinois, USA), version 23.0, was employed for analyzing the collected data. The quantitative data were displayed as mean ±SD, and ranges. Qualitative variables are shown as percentages and numbers. Shapiro-Wilk and Kolmogorov-Smirnov tests were utilized for determining whether the data were normal. The Mann-Whitney U test was utilized for comparing two groups in non-parametric data, and the Kruskall-Wallis test utilized for multiple group comparisons in non-parametric data. The Chi-square test and Fisher's exact test were utilized for comparing groups with qualitative data rather than the Chi-square test only where the predicted count in any cell was < 5, and the findings are represented by odds ratios (ORs) and 95% confidence intervals (CIs). The acceptable margin of error was set at 5%, and the CI was set at 95%. Therefore, a *p*-value of ≤ 0.05 is considered significant.

Results

The patient group consisted of 100 children diagnosed with pneumonia. They were 47 males and 53 females. The patients' mean age was 31.9±35.6 months (ranged from one to 180 months). Regarding the matched controls, there were 51 males and 49 females, their mean age was 32.4±33.6 months (ranged from three to 180 months). The description of chest X-ray and blood culture data of the patients are illustrated in Table 1

Table 1. Description of chest X-ray and blood culture data of the 100 patients.

Chest X-ray	Patients Group No (%)
Alveolar Infiltration	23 (23.0%)
Lobar consolidation	75 (75.0%)
Lobar consolidation and puemothorax	2 (2.0%)
Blood Culture	
No growth	88 (88.0%)
Streptococcus pneumoniae	2 (2.0%)
Klebsiella	5 (5.0%)
MRSA	4 (4.0%)
Pseudomonas	1 (1.0%)

Genotype and allelic frequencies of TLR2 (rs5743708) gene polymorphism of the patients and the control group

We observed that the TLR2 (rs5743708) variant in the control group was in Hardy-Weinberg equilibrium (HWE) (p=0.521) (Table 2). The most common genotype in the population under study was the wild (G/G) genotype, which was linked to a significantly decreased risk of pediatric pneumonia infection. On the other hand, 3% of those diagnosed with pneumonia infections had the mutant homozygous (A/A) genotype, which is a significant risk factor for pneumonia infections in children (p=0.045) (Table 2). In comparison to patients with wild homozygous genotypes, those with the TLR2 (rs5743708) genotype (G/A) had a 2.52 times higher risk of having pneumonia (OR: 2.52; 95% CI: 1.32-4.79; p=0.005). Also, the (A) allele of TLR2 (rs5743708) was connected to a significantly greater risk of pneumonia infection in children (OR: 1.612; 95% CI: 1.07-2.43; p=0.023) (Table 2).

Table 2. Genotype and allelic frequencies of TLR2 (rs 5743708) variant of the patients and the control groups.

TLR2 (rs5743708)	Patients Group (n=100)		Control Group (n=100)		OR (95% CI.)
	No.	%	No.	%	
Genotype					
GG	19	19.0%	38	38.0%	Reference
GA	78	78.0%	62	62.0%	2.52 (1.32-4.79), p= 0.005
AA	3	3.0%	0	0.0%	p= 0.045
Allele frequency					
G	116	58.0%	138	69.0%	Reference
A	84	42.0%	62	31.0%	1.612 (1.07-2.43), p= 0.023
Dominant model					
GG	19	19.0%	38	38.0%	Reference
GA+AA	81	81.0%	62	62.0%	2.61 (1.37-4.97), p= 0.003
Recessive model					
GG+GA	97	97.0%	100	100.0%	Reference
AA	3	3.0%	0	0.0%	p= 0.246(NS)

TLR 2: Toll-like receptor 2; No.: number; OR: odds ratio; CI: confidence interval. $p > 0.05$ is not significant (NS).

Genotype and allelic frequencies of TLR2 (rs 5743708) variant and disease severity

The mutant homozygous (A/A) genotype was observed in 2% of Subgroup I A patients (mild/moderate pneumonia) and 4% of Subgroup I B patients (severe pneumonia) (Table 3). However, this difference, in evaluating the severity of pneumonia in children, did not reach statistical significance (OR: 4.33; 95% CI: 0.32-57.65; *p*=0.267).

Table 3. Genotype and allelic frequency of the TLR2 (rs 5743708) variant in patient subgroups.

TLR2 (rs 5743708)	Subgroup IA (n=50)		Subgroup IB (n=50)		Subgroup IA vs. subgroup IB
	No.	%	No.	%	OR (CI. 95%)
Genotype					
GG	13	26.0%	6	12.0%	Reference
GA	36	72.0%	42	84.0%	2.53 (0.87-7.33), *P*=0.088 (NS)
AA	1	2.0%	2	4.0%	4.33 (0.32-57.65), *p*=0.267 (NS)
Allele frequency					
G	62	62.0%	54	54.0%	Reference
A	38	38.0%	46	46.0%	1.40 (0.79-2.44), *p*=0.252 (NS)
Dominant model					
GG	13	26.0%	6	12.0%	Reference
GA +AA	37	74.0%	44	88.0%	2.57 (0.89-7.45), *p*=0.0812 (NS)
Recessive model					
GG+ GA	49	98.0%	48	96.0%	Reference
AA	1	2.0%	2	4.0%	2.04 (0.18-23.27), *p*=0.565 (NS)

TLR 2: Toll-like receptor 2; No.: number; OR: odds ratio; CI: confidence interval, $p > 0.05$ is not significant (NS).

Association between TLR2 (rs5743708) Genotypes and Laboratory Characteristics

-Association between TLR2 (rs5743708) genotypes and laboratory characteristics in Subgroup I A:

There was a statistically significant correlation between various TLR2 genotypes and the following laboratory parameters: total leucocyte count (TLC), absolute neutrophil count, Neutrophil/Lymphocyte ratio (NLR), and CRP (*p*=0.030, *p*=0.034, *p*=0.046, *p*=0.011, respectively). There was a statistically significant correlation between various TLR2 genotypes and both CRP/Lymphocyte ratio and ESR (*p*=0.002, *p*=0.007, respectively).

-Association between TLR2 (rs5743708) genotypes and laboratory characteristics in Subgroup I B:

There was a statistically significant correlation between the various TLR2 genotypes and the following laboratory parameters: absolute lymphocyte count, absolute monocyte count and Lymphocyte/Monocyte ratio (LMR) (*p*=0.010, *p*=0.041 and *p*=0.017, respectively).

Discussion

In this study we evaluated the possible contribution of genetic variations in the TLR2 (rs5743708) variant on the risk and severity of the development of pediatric pneumonia infection. In our study, the measure of odds ratios, revealed that the rs5743708 gene variant in the TLR2 gene in dominant models (OR: 2.61; 95% CI: 1.37-4.97; *p*=0.003), heterozygote models (OR: 2.52; 95% CI: 1.32-4.79; *p*=0.005), and homozygote models (p=0.045) was related to a greater risk of pneumonia disease. Moreover, the (A) allele was found to be substantially related to a greater risk of pneumonia infection in children (OR: 1.612; 95% CI: 1.07–2.43; *p*=0.023).

Such findings are in line with the research data by the study of Gbura et al., 2017, who stated that the TLR2 gene variant Arg753Gln was associated with a negative effect on TLR2

function which reduces the nuclear factor kappa B (NF-κB) pathway's activation. This indicated a significant cellular route of both innate and adaptive immune responses and affects the expression of several genes engaged in the regulation of the main stages of immune response activation.[17]

Similarly, the study by Lorenz et al., 2000, found that the Arg753Gln variant decreases the host's immunological response by decreasing the macrophage response to bacterial peptides.[9] Additionally, the study by Xiong et al., 2012, revealed that these nonfunctional TLR2 are unable to recognize PAMPs, resulting in the failure of extracellular pathogen identification mechanisms such as Gram-negative and Gram-positive bacteria. The observed effect of this single nucleotide polymorphism (SNP) on susceptibility to pneumonia infection is linked to all of these variations in TLR2 activity.[18] In addition, the study by Lyahovskaya et al., 2013, revealed that this TLR2 gene mutation was associated with the diagnosis, treatment, and prevention of bronchial asthma in adulthood.[19]

Previous studies demonstrated a link between the TLR2 Arg753Gln polymorphism and a number of infectious illnesses, such as endocarditis, urinary tract infections, and children's recurrent bacterial infections.[20-22]

Our study showed no pronounced effect on pneumonia severity for the Arg753Gln variant of the TLR2 gene. Furthermore, we conducted subgroup analyses that compared two different subgroups of patients (non-severe, mild/moderate pneumonia patients and sever pneumonia patients). Because subgroup studies often include relatively fewer patients than the overall analysis, they are more likely to produce negative results due to insufficient power for the reduced sample size.

Inflammatory indicators, including CRP, IL-6, and ESR are commonly used to assess the severity and prognosis of pneumonia. We observed high levels of these inflammatory markers in the patient group at admission, highlighting the importance of these markers in the pulmonary inflammatory process. These results are in line with the findings of the study by Fernandes et al., 2019, who found that host biomarkers, particularly CRP, IL-6, and IL-8, have the ability to predict severe community-acquired pneumonia in pediatric populations.[23] However, our study found that there was no relationship between the inflammatory markers (CRP, IL-6, ESR) and the TLR2 (rs5743708) variant.

The relatively small number of patients in the subgroups of our study represents a significant limitation of the study. A greater sample size of patients will be required in the future to confirm the link between the TLR2 polymorphism (rs5743708) and the severity of pediatric pneumonia.

In conclusion, the TLR2 (rs5743708) mutant (G/A) genotype and the (A) allele were correlated to an increased risk of pneumonia infection in children. However, they did not represent an important risk factor for severity of infection.

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Author Contributions

SMA, conception; design of the work; analysis; interpretation of data; writing the original draft. AAA, laboratory investigations; methodology; acquisition of data; analysis; supervision. ENAM, SMK, examination; data collection and analysis; supervision. EMM, manuscript drafting, and revision. WHA, AKA, examination; revising the manuscript. AAE, statistical analysis; manuscript revision. All authors have read and approved the manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. (Approval No. 2023011676).

Informed consent

An informed written consent was obtained from one parent of each study participant before included in the study.

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