J Appl Biotechnol Rep. 2026 March;13(1):1972-1981

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2026.574287.1982

Original Article

The Impact of Genetic Variations on Immune Dysregulation and Inflammatory Response in Pneumonia

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Received February 6, 2026; **Accepted** February 17, 2026; **Online Published** March 30, 2026

Abstract

Introduction: Pneumonia remains an important medical issue, associated with various illnesses and deaths, and is strongly dependent on host defense mechanisms. Polymorphisms in immune-regulating genes may affect the production and secretion of certain cytokines, thereby increasing the inflammatory response during an infection. This study aimed to establish the association between inflammatory cytokines, immune-related polymorphisms, and inflammatory marker expression in patients with pneumonia.

Materials and Methods: One hundred twenty-two patients with pneumonia and thirty healthy controls were enrolled at Baghdad Medical City Teaching Hospital from March to August 2025. Their serum cytokine and inflammatory marker levels were determined using ELISA. Polymorphisms in the genes *IL6*, *IL10*, *TLR4*, *NLRP3*, *IFN-γ*, and *CRP* were studied using PCR and qPCR.

Results: Pneumonia patients (n = 122) had significantly increased IL-6 (118.4 ± 35.7 vs. 16.2 ± 5.9 pg/ml), TNF-α (92.5 ± 28.6 vs. 14.8 ± 4.5 pg/ml), and CRP levels (76.2 ± 20.5 vs. 6.1 ± 3.0 mg/L) compared to controls (n = 30; all $p < 0.001$) and negative correlations between these mediators and IL-10 (r = -0.45).

Conclusions: The immune and inflammatory responses in the patients' bodies are shown to become extensively unregulated and overactive as a result of IL6 and TLR4 cytokine expression and production, which are directly related to the severity of pneumonia.

Keywords: Pneumonia, Immune Response, Inflammation, Cytokines, Polymorphism

Citation: Hamad ON, Saddam NK, Hamad MN. The Impact of Genetic Variations on Immune Dysregulation and Inflammatory Response in Pneumonia. J Appl Biotechnol Rep. 2025;13(1):1972-1981. doi:10.30491/jabr.2026.574287.1982

Introduction

Pneumonia remains among the most common causes of infectious deaths globally, with an annual mortality of approximately 2.5 million, and is also a major pulmonary complication in patients with cardiometabolic comorbidities.[1,2] While advances in antimicrobial stewardship and vaccination have contributed to reductions in case fatality rates in high-resource settings, the situation for Low- and Middle-Income Countries (LMICs), especially those within the Middle East, is discouraging when it comes to mortality due to pneumonia, which is higher than the global average.[3-5] Recent evidence has proposed that the clinical variability in pneumonia is not only due to differences in pathogen virulence but also to differences in the immune response of the host, whereas immunogenetics determine susceptibility and disease outcome.[6]

Pro-inflammatory cytokines such as IL-6 and TNF-α coordinate leukocyte trafficking, endothelial activation, and hepatic acute-phase response through the induction of C-reactive protein (CRP), with counter-regulatory negative feedback restraint via IL-10.[7] Meta-analyses demonstrate that high levels of IL-6 (>100 pg/ml) and TNF-α are associated with greater oxygen needs, the need for ICU, and mortality in pneumonia cohorts.[8] Immune-regulatory polymorphisms vary extensively between ethnic groups in both allele frequency and functional effect.[9,10]

SNPs in IL6 (−174 G/C; rs1800795),[11] TLR4 (A896G; rs4986790),[12] IL10 (−1082 A/G; rs1800896),[13] NLRP3 (Q705K, SNP rs35829419),[14] IFN-γ (+874 T/A; rs2430561),[15] and CRP (rs1205)[16] have been shown to alter the transcriptional activity or function of their respective receptors, which in turn modifies cytokine response during an infection. Despite studies focusing on specific variants in European and Asian cohorts, there remain three major constraints: (i) Fragmented analysis of individual genes overlooks polygenic effects that control immune networks; (ii) lack of concomitant cytokine profiling precludes meaningful genotype–phenotype correlation mapping; and (iii) the situation emerges as a virtual desert for Iraqi populations while ethnic divergence has been reported for the architecture of immune-related gene loci.[17]

This study addresses these gaps with three contributions. Firstly, it is the first presentation of a seven-gene immune-regulatory polymorphism panel in a well-defined Iraqi pneumonia patient population, and this information is key for clinical application. The second reason is the fact that we integrate multiplex genotyping with a quantitative profiling of the cytokine network (IL-6, TNF-α, IL-10, CRP) in a descriptive manner of functional immune dysregulation disequilibrium pathways rather than single risk alleles, an approach not launched by previous regional studies. Third, it establishes clinically actionable genotype-phenotype associations that might inform risk stratification for hyperinflammation in a population without precision medicine frameworks for respiratory infection. We sought to test the hypothesis that (i) IL6 -174 G and TLR4 896G alleles would predispose to an enhanced cytokine response to pathogens and susceptibility to pneumonia; (ii) IL10 -1082 G would be associated with a more anti-inflammatory phenotype of counter-regulation; and (iii) NLRP3 Q705K with certain phenotypes of inflammasome activation. Integrating population genetics with cytokine network analysis, the study establishes a first blueprint for genotype-dependent immunomodulation strategies in an understudied high-burden setting.

Materials and Methods

Study Design and Population

The genetic polymorphisms of immune regulatory genes in patients with pneumonia and their inflammatory responses were explored using a case-control approach. From March 8 to August 22, 2025, the research was conducted at one of the largest multi-specialty hospitals in the region, the Baghdad Medical City Teaching Hospital. The study was compliant with the 2013 Declaration of Helsinki. Written and informed consent was obtained from participants. Ethical approval was obtained from the Ethics Review Committee at Baghdad Medical City Teaching Hospital. In total, the study enrolled 122 patients with pneumonia and 30 age- and gender-matched controls. All participants underwent clinical, imaging, and laboratory assessments to diagnose pneumonia.

Inclusion Criteria

Volunteers aged 18 years and above with pneumonia who were willing to participate in a detailed genetic and biochemical assessment were recruited. Patients with advanced pneumonia who exhibit acute respiratory symptoms and lack a clinical history of autoimmune disorders, primary or secondary cancers, or chronic inflammatory diseases also fit the criteria. The reports indicated that the patients were not chronic users of any anti-inflammatory or immunosuppressive agents and were not affected by any immune or inflammatory disorders.

Exclusion Criteria

Asthmatics, patients with tuberculosis, and those with chronic obstructive pulmonary disease (COPD) were not included in the study sample. Exclusion criteria included patients with malignancies, autoimmune diseases, chronic infections, and those on corticosteroids, immunosuppressants, or prolonged anti-inflammatory therapy. Other exclusion criteria included patients on antibiotics before admission, pregnant or lactating women, and those unable to provide an adequate sample volume or who did not provide a properly signed informed consent form.

Sample Collection and DNA Extraction

For this study, each participant had 5 ml of peripheral blood drawn and placed in EDTA-coated blood collection tubes. The participants' blood samples were transported under specific temperature conditions for storage. All samples were collected within 2 hours. According to the protocols, the Qiagen DNA Mini Kit (Qiagen, Germany) was used to isolate the genomic DNA. The sample was quantified, and its quality and purity were evaluated by measuring absorbance with a spectrophotometer. The DNA sample was also assessed by 1% agarose gel electrophoresis and subsequently stained with ethidium bromide. Additionally, the sample underwent confirmatory tests using agarose gel electrophoresis. The purified DNA samples were stored at -20 °C for later use in processes such as polymerase chain reaction (PCR) amplification, genotyping, and cytokine expression assays. Additionally, for assessment of cytokine levels the ELISA method was used.

Target Genes and PCR Amplification

The immune-regulatory and inflammation response genes chosen in response to pneumonia and focused on in this study were *IL6*, *TNF*-α, *IL10*, *TLR4*, *NLRP3*, *IFN-γ*, and *CRP*. Some portions of these genes with polymorphisms were selected from the literature for biological significance. Amplification of the target regions of the genes was performed using the PCR with primers designed and deposited in GenBank (Table 1).

The PCR was carried out in a final volume of 25 µl, which included the following components: 50 ng of gDNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, and 1 unit of Taq DNA polymerase (Thermo Fisher Scientific, USA). Each of the 35 cycles of amplification was set at 94 °C (denaturation), 57-60 °C (depending on the primers), and 72 °C (extension), with a final extension of 5 minutes at 72 °C for each set of samples. The samples underwent 2% PCR electrophoresis. Ethidium bromide was used to stain them, and they were examined for the correct size amplicons. Table 2 lists the most important polymorphisms investigated in this study due to their functional modulation of immune responses and inflammatory

signaling during pneumonia.

The polymorphisms of *IL6* (–174 G/C; rs1800795) and *TNF*-α (–308 G/A; rs1800629) gene cytokines are located in the promoter regions of these genes and are known to modify transcriptional activity, thereby altering circulating levels of the inflammatory response augmenters IL-6 and TNF-α. The IL-10 (–1082 A/G; rs1800896) polymorphism is located in the IL-10 promoter; hence, there is a balancing act in the modulation of pro- and anti-inflammatory signaling that orchestrates the response. The TLR4 (A896G; rs4986790) polymorphism, associated with the primary structure of a protein (Asp299Gly) alteration, is the result of a change that impedes the recognition of LPS (lipopolysaccharide) and, in the most extreme case, may block the recognition of other pathogens and the activation of NF-κB. Likewise, the NLRP3 (Q705K; rs35829419) variant, which encodes a missense mutation, can further stimulate the inflammasome to elicit IL-1β and, to a far lesser extent, is associated with unregulated inflammation. The polymorphism of IFN-γ (+874 T/A; rs2430561) in intron 1 is known to augment the production of IFN-γ, along with enhancing the responses of Th1 cells. The CRP polymorphism 1059G>C (rs1205) is known to modify plasma CRP levels, a marker of systemic inflammation. To summarize, the polymorphic loci presented in this study function within a defined network of genetic elements that control cytokine expression and the inflammatory response during pneumonia.

Table 1. PCR Primer Sequences and Amplicon Characteristics

Gene	Primer sequence (5′ → 3′)	Amplicon size (bp)	Annealing temp (°C)	Genomic position	Reference
IL6	F: GGTACATCCTCGACGGCATCT R: GTGCCTCTTTGCTGCTTTCAC	198	58	chr7:22727027–22727225	(18)
TNF-α	F: GAGGCAATAGGTTTTGAGGGCCAT R: GGGACCTCTCTCTAATCAGCCCT	215	60	chr6:31543012–31543227	(19)
IL10	F: CCTAGGTCACAGTGACGTGG R: GATGTCAAACTCACTCATGGCT	175	57	chr1:206946549–206946724	(13)
TLR4	F: TTAGGCTGAGTTTCTGCAACCT R: TCCATCCAGGAGGAAAGAAA	223	59	chr9:117713019–117713242	(20)
NLRP3	F: TGTGCTGAGTTCCCTGTGAC R: CTCTTGTTCTCGGGTCCATC	201	60	chr1:247588348–247588549	(14)

Table 2. Reference Single-Nucleotide Polymorphisms (SNPs) of Immune-Regulatory Genes Analyzed in Pneumonia Patients

Gene	Polymorphism	dbSNP ID (rs#)
IL6	–174 G/C	rs1800795
TNF-α	–308 G/A	rs1800629
IL10	–1082 A/G	rs1800896
TLR4	A896G	rs4986790
NLRP3	Q705K	rs35829419
IFN-γ	+874 T/A	rs2430561
CRP	1059G>C (C/T)	rs1205

Statistical Analysis

All analyses of the data were conducted using IBM SPSS Statistics, version 27, and R, version 4.3. Continuous variables are reported as mean ± standard deviation (SD), and categorical variables are reported as frequencies and proportions. The Shapiro–Wilk test was used to assess the normality of the data. Genotype and allele frequencies were counted directly, and the Hardy-Weinberg equilibrium (HWE) was tested using the chi-square test. Subjects were grouped as patients and controls and analyzed using an independent-samples t-test or a Mann–Whitney U test, as appropriate. To assess the association between genotypes and cytokine levels, one-way ANOVA or the Kruskal–Wallis test was performed, followed by post hoc analyses with the Bonferroni correction. Relationships were evaluated using Pearson's or Spearman's correlation coefficients between the cytokines (IL-6, TNF-α, and IL-10) and inflammatory parameters (CRP and WBC), as well as the polymorphic genes. The association between genetic markers and susceptibility to pneumonia was assessed using odds ratios (ORs) with 95% confidence intervals (CIs) from binary logistic regression, adjusted for age and sex confounders. For all analyses, a p-value of 0.05 was used as the significance threshold.

Results

Demographic and Clinical Characteristics

In Table 3, the clinical and demographic attributes of the subject population are described. It was found that the participant means and distributions of age and sex did not differ by cohort ($p > 0.05$), indicating that the cohorts are well-matched and that demographic bias has been minimized. Patients with pneumonia had significantly elevated levels of CRP, WBC, and other important inflammatory cytokines, including IL-6, TNF-α, and IL-10, compared with healthy controls ($p < 0.001$ for each), demonstrating systemic inflammation.

In this sample, patients had longer average fever

durations and greater illness and hospitalization, suggesting a more severe infection. A concerning 42.6% of participants were chronic, habitual smokers, and this likely played a role in the deficiency of the immune system and cytokine storm that was observed. Patients and controls also did not differ in average body mass index, which indicates that body fat was not a confounding factor that could have biased the findings. The 7.4% case fatality rate for pneumonia observed in the sample was particularly astonishing, underscoring the impact of such an inflammatory and genomic inquiry. Compared with controls, patients with pneumonia had significantly higher serum levels of IL-6, TNF-α, and IL-10 ($p < 0.001$). Regarding IL-6 levels, patients had markedly elevated levels of 118.4 ± 35.7 pg/ml, whereas the control group had barely detectable levels of 16.2 ± 5.9 pg/ml. Furthermore, although the patients tested positive for TNF-α and IL-10, their average IL-10 levels were 92.5 ± 28.6 pg/ml and 28.6 ± 8.9 pg/ml, respectively, which were lower than expected. These data point to the existence of substantial, 'active' pro- and anti-inflammatory networks in pneumonia.

Table 3. Demographic and Clinical Characteristics

Variable	Pneumonia patients (n = 122)	Controls (n = 30)	*p*-value
Age (years, mean ± SD)	54.7 ± 13.1	52.9 ± 11.4	0.287
Sex (Male %)	61 %	58 %	0.644
Body Mass Index (kg/m)	27.6 ± 3.9	26.8 ± 3.4	0.319
Smoking history (%)	42.6 %	26.7 %	0.048
Duration of illness (days)	7.4 ± 2.3	—	—
Fever (>38 °C, %)	83.6 %	10.0 %	<0.001
C-Reactive Protein (CRP, mg/L)	76.2 ± 20.5	6.1 ± 3.0	<0.001
White Blood Cell Count ($\times 10^9$/L)	12.8 ± 3.9	6.4 ± 1.7	<0.001
IL-6 (pg/ml)	118.4 ± 35.7	16.2 ± 5.9	<0.001
TNF-α (pg/ml)	92.5 ± 28.6	14.8 ± 4.5	<0.001
IL-10 (pg/ml)	28.6 ± 8.9	12.3 ± 3.2	<0.001
Hospitalization duration (days)	8.6 ± 2.5	—	—
Mortality outcome (%)	7.4 %	0 %	0.041

Cytokine Quantification

To assess the extent of pneumonia in patients and their corresponding controls, the primary focus was on cytokine quantification and expression. In addition, to evaluate the effectiveness of primers used to amplify specific target genes in qPCR, the target genes were limited to *IL6*, *TNF*-α, *IL10*, *TLR4*, and *NLRP3* (Figure 1).

To understand the phenotype expression in relation to elevated cytokine levels and the consequent inflammation, phenotype-specific serum cytokine measurements were used. To validate the control and pneumonia cDNA samples for immune genes, qPCR was performed, and the results are shown in Figure 2. This was a refinement exercise to meet the expression analysis goals, and the defined target sequences were confirmed.

Serum IL-6 and TNF-α, along with IL-10, are categorized into three genotypes among participants: GG, GC, and CC. GG has the highest median concentrations of IL-6 and TNF-α, suggesting a stronger pro-inflammatory response. In contrast, CC has lower cytokine levels, the highest IL-10 concentration, and the highest expression across the three genotypes. The statistical differences are indicated with asterisks (*) and have a *p*-value of 0.05.

Profiles and melt curves of *IL-6, TNF-α, IL-10, TLR4*, and

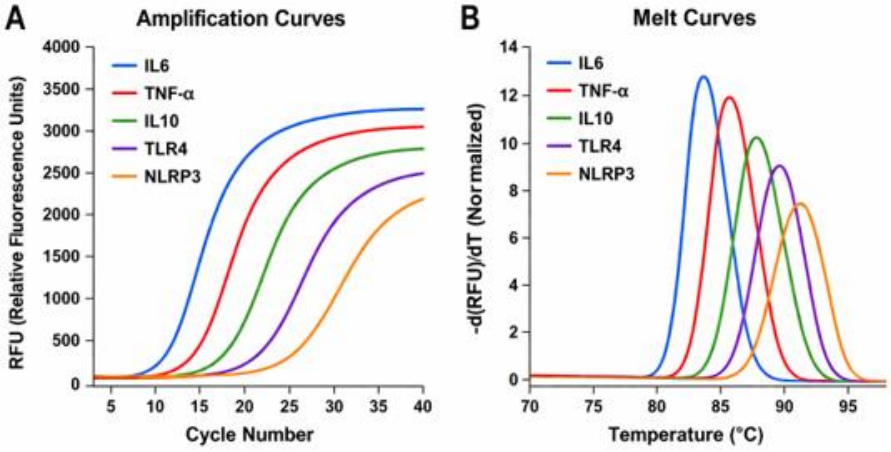

Figure 1. Using Quantitative PCR for Amplification of Immune-Associated Genes and Examining the Melting Curves. The amplification curves for the PCR process (IL6, TNF-α, IL10, TLR4, and NLRP3) are shown in Panel A and indicate exponential amplification over 25-30 cycles. Panel A illustrates the curves and the melting phase; each curve is represented by one notable peak, which indicates primer dimer or nonspecific product, and the "melt" curve confirms primer specificity. Such a final affirmation means that the primers designed for the specimens with pneumonia gene expression were indeed appropriate.

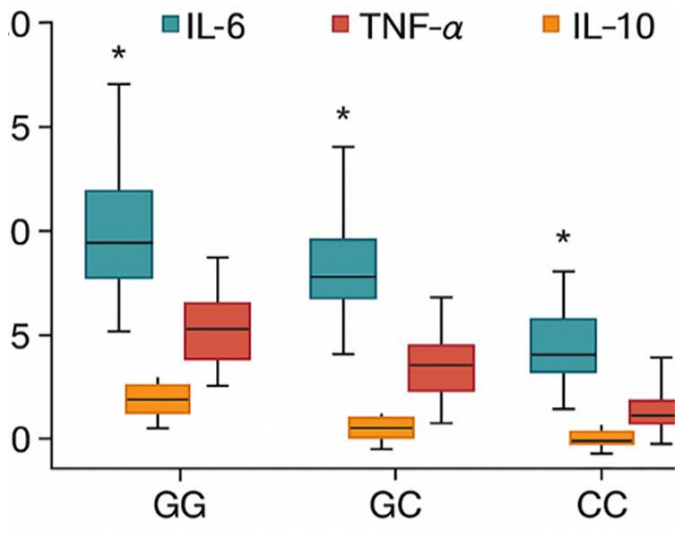

Figure 2. The Boxplots Show the Serum Levels of Cytokines in IL6 (–174 G/C) Polymorphism Carriers.

NLRP3 genes were used to assess amplification and define relative target expression in the PCR products, which, in turn, allowed us to evaluate primer effectiveness, reaction efficiency, and product certainty. We analyzed the effect of genetic polymorphism on cytokine expression by measuring serum levels of IL-6, TNF-α, and IL-10 in pneumonia patients with different IL-6 (-174 G/C) genotypes. This study aims to investigate whether a specific polymorphism in the *IL6* gene affects the balance between pro- and anti-inflammatory cytokine responses at the cellular level. Genotype comparisons within the box plots of Figure 3 illustrate changes in cytokine concentration and the phenotypic impact of variation in the *IL6* gene.

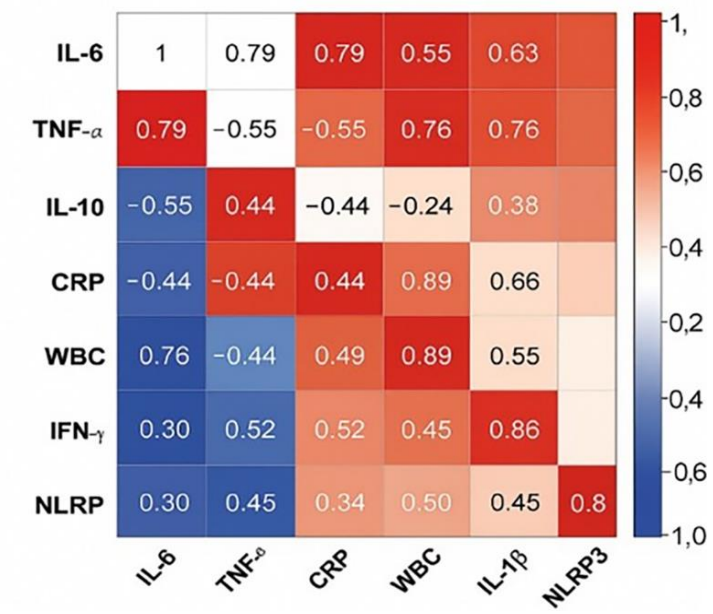

Figure 3. The Correlation Heatmap of Inflammatory and Immune Parameters in Pneumonia Patients.

Genotype and Allelic Frequencies

The differences in the distribution of the IL6–174 G/C and TLR4 A896G polymorphisms between the patient population and a group of healthy controls have been shown to result from a genetic predisposition to infection. The increased IL6 GG genotype in the patient population supports the notion of a stronger correlation between IL-6 signaling and the inflammatory genotypes of this class. In addition, TLR4 A896G patients had a higher proportion of AG/GG genotypes, likely reflecting an increased immune response to bacterial inflammation. In another instance, polymorphisms of IL10, NLRP3, IFN-γ, and CRP did not provide significant differences between the two cohorts. In conclusion, the data indicate that IL6 and TLR4 methylation and coding polymorphisms are relevant to the innate immune response to pneumonia. Observed differences in the distributions of genotypes and the frequencies of alleles for polymorphic immune-regulating genes were found in patients with and without

infections, as well as in patients with infections. More details about these immune polymorphisms can be found in Table 4.

The entire sample set was found to be in Hardy-Weinberg equilibrium (HWE) ($p < 0.05$) for all markers, indicative of adequate sampling population diversity as well as the accuracy of genotype coding. The two groups under study differed with respect to polymorphisms in the *IL6* (rs1800795), *TNF*-α (rs1800629), and *TLR4* (rs4986790) genes. Observed differences in the distributions of genotypes and the frequencies of alleles for polymorphic immune-regulating genes were found in patients with and without infections, as well as in patients with infections. More details about these immune polymorphisms can be found in Table 4. The amplification curves for the PCR process (*IL6, TNF-α, IL10, TLR4,* and *NLRP3*) are shown in Panel A and indicate exponential amplification over 25-30 cycles. Panel B illustrates the curves and the melting phase; each curve is represented by one notable peak, which indicates primer dimer or nonspecific product, and the "melt" curve confirms primer specificity. Such a final affirmation means that the primers designed for the specimens with pneumonia gene expression were indeed appropriate.

Table 4. Genotype and Allelic Frequencies of Immune-Regulatory Gene Polymorphisms in Pneumonia Patients and Controls

Gene (SNP)	Genotype	Pneumonia patients (n = 122)	Controls (n = 30)	Allele frequency (Patients)	Allele frequency (Controls)	HWE *p*-value	*p*-value (case vs control)
IL6 (rs1800795)	GG / GC / CC	70 (57.4%) / 35 (28.7%) / 17 (13.9%)	6 (20.0%) / 12 (40.0%) / 12 (40.0%)	G = 72%	G = 40%	0.81	0.01*
TNF-α (rs1800629)	GG / GA / AA	62 (50.8%) / 45 (36.9%) / 15 (12.3%)	20 (66.7%) / 8 (26.7%) / 2 (6.6%)	G = 69%	G = 80%	0.74	0.048*
IL10 (rs1800896)	GG / GA / AA	50 (41.0%) / 60 (49.2%) / 12 (9.8%)	9 (30.0%) / 15 (50.0%) / 6 (20.0%)	G = 66%	G = 55%	0.65	0.19
TLR4 (rs4986790)	AA / AG / GG	90 (73.8%) / 25 (20.5%) / 7 (5.7%)	28 (93.3%) / 2 (6.7%) / 0 (0%)	A = 84%	A = 96%	0.87	0.03*
NLRP3 (rs35829419)	CC / CK / KK	85 (69.7%) / 30 (24.6%) / 7 (5.7%)	25 (83.3%) / 4 (13.4%) / 1 (3.3%)	C = 82%	C = 90%	0.90	0.11
IFN-γ (rs2430561)	TT / TA / AA	40 (32.8%) / 58 (47.5%) / 24 (19.7%)	11 (36.7%) / 13 (43.3%) / 6 (20.0%)	T = 57%	T = 58%	0.76	0.84
CRP (rs1205)	CC / CT / TT	55 (45.1%) / 50 (41.0%) / 17 (13.9%)	13 (43.3%) / 13 (43.3%) / 4 (13.4%)	C = 66%	C = 65%	0.92	0.96

Association between Genetic Variants and Cytokine Levels

In most scenarios presented in Table 5 and other tables, the gaps in genotype distributions were so large that they indicated the population was carrying genes that, in combination with inherited immune genes, can control cytokine production and inflammation ($p < 0.05$).

Table 5. Association between Gene Polymorphisms and Cytokine Levels in Pneumonia Patients

			Cytokine levels			
Gene (SNP)	Genotype	n	Mean IL-6 (pg/ml) ± SD	Mean TNF-α (pg/ml) ± SD	Mean IL-10 (pg/ml) ± SD	*p*-value
IL6 (rs1800795)	GG	70	120.6 ± 34.2	85.3 ± 27.4	25.1 ± 8.2	0.02
	GC	55	98.5 ± 29.8	73.6 ± 22.1	27.8 ± 8.7	
	CC	25	88.4 ± 27.5	65.8 ± 19.3	30.5 ± 9.4	
IL10 (rs1800896)	GG	50	109.2 ± 32.7	80.6 ± 25.8	31.8 ± 9.6	0.01
	GA	60	102.4 ± 29.5	74.2 ± 23.7	27.4 ± 8.8	
	AA	30	93.6 ± 25.9	69.1 ± 22.4	22.8 ± 7.5	
TLR4 (rs4986790)	AA	90	118.3 ± 33.8	86.9 ± 26.5	27.2 ± 8.9	0.03
	AG	40	101.6 ± 28.4	73.5 ± 23.9	25.9 ± 8.4	
	GG	22	88.9 ± 26.3	66.8 ± 20.1	24.7 ± 7.6	
NLRP3 (rs35829419)	CC	85	107.8 ± 31.5	79.6 ± 25.4	26.8 ± 8.1	0.04
	CK	50	117.4 ± 34.1	83.9 ± 26.2	28.1 ± 8.5	
	KK	17	132.6 ± 37.2	91.5 ± 29.0	30.4 ± 9.2	

The highest concentrations of IL-6 and TNF-α were observed in the subgroup with the IL6 (rs1800795) GG genotype, suggesting that the IL-6 GG genotype is most likely transcriptionally active and increases the pro-inflammatory response. In contrast, the pro-inflammatory cytokines of the CC genotype were markedly lower, suggesting that the CC genotype is the most dominant pro-inflammatory cytokine genotype. Among IL-10 GA and AA genotypes, the IL-10 (rs1800896) GG genotype was associated with the highest IL-10 levels, suggesting that, compared to the others, this genotype enhances the response to deficient anti-inflammatory cytokines.

For the polymorphism of TLR4 (rs4986790), the AA genotype of the individuals had median values of IL-6 and

TNF-α, which were higher compared to the other two TLR4 polymorphisms (AG and GG), suggesting greater TLR immune pathway activation. Likewise, the NLRP3 (rs35829419) KK variant was reported to have the highest levels of the inflammatory cytokines IL-6 and TNF-α, suggesting the ability to promote inflammation and IL-1β activity.

Correlation between Cytokines and Inflammatory Markers

In Figure 3, the correlation matrix of inflammatory and immune markers in patients with pneumonia shows several relationships, with a balance of pro- and anti-inflammatory activities. IL-6, TNF-α, and CRP had strong positive intercorrelations, suggesting cytokine and acute-phase inflammation activities were triggered together. IL-1β was dependent on NLRP3 and associated with NLRP3-driven inflammation, supporting the hypothesis of inflammasome control dominance. IL-10, an anti-inflammatory cytokine, showed moderate inverse correlations with IL-6, TNF-α, and CRP, suggesting that IL-10 acts as an anti-inflammatory rather than a synchronising factor after uncontrolled inflammation. IL-10 correlations with IFN-γ were moderate and bidirectional, indicating an influence on both the innate and the adaptive immune systems.

The heatmap shows the correlations among IL-6, TNF-α, IL-10, CRP, WBC, IFN-γ, IL-1β, and NLRP3 using correlation matrices, where positive correlations are depicted in red and negative correlations in blue, with intensity indicating the strength of the correlation (from 1 to +1). The most significant positive correlations were observed between IL-6 and TNF-α, CRP, IL-1β, and NLRP3, suggesting the potential simultaneous activation of inflammatory pathways. In contrast, IL-10 showed moderate negative correlations with the principal pro-inflammatory factors, suggesting that IL-10 dampens the immune response.

Discussion

The average age of patients with pneumonia in this study was 55 years. The majority of patients were male, accounting for approximately 61% of the study population. This corroborates with existing epidemiological research, which indicates that middle-aged and older individuals suffer from severe pneumonia due to immune suppression and various other comorbidities.[21,22] The age and sex distributions of cases and controls are matched, thereby minimising selection bias and enhancing the validity of subsequent immunogenetic evaluations. The studies by Rosenthal et al.,[4] and Günen et al.[23] provided more detailed insights concerning the older male population with pneumonia in Asian and Middle Eastern countries.

Patients had distressingly elevated mean levels of CRP, IL-6, TNF-α, and IL-10 compared with controls ($p < 0.001$). This indicates the presence of pneumonia, which is commonly associated with bacterial and viral entities. The elevation of CRP and WBC is characteristic of the inflammatory response, driven by IL-6 and orchestrated by the NF-κB signaling pathway. This inflammation is noted by Zhao et al.[24] and Zhang et al.,[25] which demonstrated that CRP and IL-6 are the most important diagnostic biomarkers for severe pneumonia. Furthermore, Zhu et al.[26] noted that the simultaneous increase in CRP and IL-6 enhances diagnostic differentiation, particularly in bacterial pneumonia and sterile inflammation.

A rise in IL-6, TNF-α, and IL-10 levels simultaneously indicates both the presence of inflammation and its resolution. A study by Carlini et al.[27] reported on these cytokines and noted that the increase in IL-10 is part of an anti-inflammatory response aimed at counteracting hyper-inflammation. Another study by Martinez-Espinosa et al.[28] indicated that IL-10 is protective against immune-mediated host damage and, under restrictive conditions, can also protect the host from infection during clearance. The cytokines from Baghdad Medical City Teaching Hospital reflect severe respiratory infections, predominantly caused by bacteria.

There is a direct relationship between smoking and the severity of pneumonia, illustrating the deleterious effect of smoking as a modifiable inflammatory risk factor. Smoking is known to induce oxidative stress and dysregulation of cytokines, increasing the risk of a hyperinflammatory response in the lungs. Increased levels of IL-6 and TNF-alpha in pneumonia smokers and in those with prolonged hospitalization were also reported by Liu et al.[20]

In that population, the increased prevalence of fever (83.6%) and the length of hospital stay (8.6 ± 2.5 days) were driven by an enhanced inflammatory response. This aligns with the work of Popadic et al.,[29] who established correlations between cytokine levels and oxygen demand, as well as between oxygen demand and clinical outcomes. The 7.4% mortality rate in this study was similar to that reported in multicentre pneumonia studies in the Middle East.[4] Mortality has been associated with prolonged elevation of IL-6, TNF-α, and CRP, which results in endothelial dysfunction and respiratory failure. These results certainly demonstrate that hyperinflammation has a strong impact on the prognosis and encompass the genetic factors that will be elaborated in the next sections.

Using cytokine assays, it was found that pneumonia was associated with an overproduction of IL-10, along with IL-6 and TNF-α. In this scenario, IL-10 secretion is considered a key factor in the development of pneumonia. Increased levels of IL-6 indicate the activation of the NF-κB–STAT3 signaling pathway, while TNF-α suggests stimulation of macrophages and T-cells. These results are consistent with those of Tian et al.,[30] who noted that pneumonia patients with severe inflammation of IL-6 and TNF-α required longer

hospital stays. The elevation of IL-10 in this context is likely an attempt to reduce inflammatory responses, as suggested by Martinez-Espinosa et al.[28] However, the overexpression of IL-10 may be detrimental as it could lead to secondary infections, as concluded by Mikhailova et al.[31]

Genotype-based analyses revealed that patients with the IL6 (–174 GG) genotype had significantly higher IL6 and TNF-α levels than GC or CC carriers, supporting the claimed increase in transcriptional activity associated with this promoter variant. Equivalent genotype–phenotype correlations were described by Rosenthal et al.[4] On the other hand, IL10 (–1082 GG) genotype patients had the highest circulating IL-10 levels, confirming its contribution to IL-10 production.[13] The qPCR validation (Figure 2) demonstrated the assay's high specificity, capturing true biological differences rather than methodological variance.[32]

The boxplot analysis of IL-6 GG carriers (Figure 3) revealed that patients with the CC genotype had higher IL-10 levels, suggesting that IL-10 acts as a regulatory cytokine. Pro-inflammatory phenotypes, such as IL-6 and IL-10, were described by Mińko et al.[33] in the context of COVID-19 pneumonia, and CRP levels are regulated by the joint action of IL-6 and IL-10,[29] suggesting an inflammation-resolving mechanism. In general, these findings support the premise that combined cytokine alterations are a consequence of both genetic factors and environmental influences and highlight the predictive value of cytokine ratios rather than individual markers.[32]

The analysis reveals differences in the IL6 (–174 G/C) and TLR4 (A896G) variants between patients and controls, suggesting that polymorphic IL6 and TLR4 genotypes may confer genetic risk for this form of pneumonia, along with other previously listed variants. IL10 (–1082 A/G), NLRP3 (Q705K), IFN-γ (+874 T/A), and CRP (rs1205) variants did not show significant differences, but they may influence immune tone. The high prevalence of the IL6 GG genotype in patients (57.4%) may indicate stronger transcriptional activity, leading to dominant IL-6 and TNF-α expression, as demonstrated in this and other studies by Braga et al[13] and Rosenthal et al.[4]

The TLR4 A896G (Asp299Gly) polymorphism also shows significant genotypic differences between groups, suggesting defective recognition of bacterial LPS and altered cytokine signaling. This variant was described as a risk allele for respiratory infections by Mikhailova et al.[31] and as associated with diminished TLR4 expression in viral pneumonia by Mińko et al.[33] These studies strengthen the notion that IL6 and TLR4 polymorphisms may increase the risk of pneumonia by disrupting the balance of the innate and adaptive immune systems.

The variants of IL10, NLRP3, and IFN-γ had a weak effect on cytokine balance, not on susceptibility. Zhang et al.[25] and Tian et al.[30] have also found that IL10 variants regulate cytokine balance. The NLRP3 Q705K variant, although not significant, is functionally associated with enhanced IL-1β secretion.[32] The study by Chen et al.[34] on the geography of genotype frequencies examines the impact of ancestry on the evolution of immune genes. All these data suggest a polygenic model in which variants in IL6 and TLR4 are the main drivers of susceptibility, while IL10 and NLRP3 are modulators of the immune response.

The imbalance in cytokines in pneumonia also correlates with genetic variants, further emphasizing the heritable components of dysregulated immunity. Carriers of the IL6–174 GG genotype have been found to have higher levels of IL-6 and TNF-α, in line with Rosenthal et al,[4] who found that GG genotype carriers have stronger NF-κB activation and higher cytokine levels. Also, the IL10–1082 GG polymorphism was associated with high levels of IL-10, consistent with its anti-inflammatory action, but also reflects its capacity to impede the clearance of pathogens.[28]

The TLR4 A896G variant increases IL-6 and TNF-α levels, despite reduced LPS sensitivity. This is most likely due to compensatory inflammasome activation or an 'autoinflammatory lesion' scenario.[33] In the case of the NLRP3 Q705K variant, the association is even stronger, as it correlates with increased levels of active inflammasome components driven by IL-1β and IL-6.[32] This promptes cascading autoregulation of cytokine and immune circuits, with promoter and receptor polymorphisms likely orchestrating this process. The positive and negative co-associations observed with IL-6, TNF-α, and CRP, as well as with IL-10, further suggest a polygenetic interplay among these variants.[29]

The analysis of correlation indicated a significant positive association for IL-6, TNF-α, and CRP, while the ratios of these mediators to IL-10 were negative. NLRP3 and IL-1β exhibited a strong correlation, providing additional evidence for the inflammasome's role in cytokine maturation. The IL-6-CRP association presented here is also the same as in Xu et al.,[35] in which IL-6 was shown to stimulate the expression of liver CRP through a protein pathway involving the cell nuclear factor STAT3. A study by Carlini et al.[27] also stated that coelevation of IL-6 and CRP predicts longer hospitalization and higher mortality.

The works of Tian et al[30] and Mikhailova et al.,[31] which detail the combined effects of IL-6 and TNF-α on inflammation and vascular injury, are further corroborated by the strong positive correlation between these two factors. Also, the works of Surabhi et al.[36] demonstrated that NLRP3-dependent IL-1β release amplifies these cytokines, thereby establishing a self-perpetuating inflammatory cycle. On the contrary, the correlation of IL-10 with IL-6 and TNF-α is negative and indicates that, unlike other inflammatory cytokines, IL-10 is NF-κB inflammation-suppressing and responsive compensatory.[28] The interplay is more succinctly

captured in a heatmap (Figure 3). It echoes the work of Mińko et al.[33] showing the inflammatory cores of IL-6, TNF-α, IL-1β, and CRP, with IL-10 as the counterbalancer. This pneumonia immunopathology, with its intricate inter-cytokine network, underscores cytokine correlation profiling as a candidate for diagnostic and prognostic biomarkers.

Conclusion

This study greatly affirms the fact that the immune-regulatory genetic polymorphisms related to patients, particularly cytokine expression and inflammation balance, are underinvestigated. These polymorphisms are present in patients attending Baghdad Medical City Teaching Hospital and are associated with inflammatory responses. Specifically, polymorphisms of IL6 (–174 G/C) and TLR4 (A896G) are linked to higher plasma levels of IL-6, TNF-α, and CRP, respectively. On the other hand, the IL10 polymorphism (–1082 A/G) is associated with increased IL-10 expression. Although not statistically significant, genotypes of NLRP3 (Q705K), IFN-γ (+874 T/A), and CRP (rs1205) seem to interact within cytokine correlation networks, indicating weak control. This study's focused analysis of intra-phenotype and genotype correlations lays the groundwork for the development of predictive biomarkers for hyper-inflammation. Future research should expand on this study by implementing a comprehensive, ongoing approach to the genomic-cytokine field to explore its therapeutic potential.

Authors' Contributions

ONH: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization; NKS: Resources, Investigation, Laboratory analysis (genotyping and cytokine assays), Validation, Writing – review & editing; MNH: Project administration, Patient recruitment and clinical assessment, Ethical compliance oversight, Writing – review & editing. All authors approved the final version of the articles.

Ethical Approval

In accordance with the Declaration of Helsinki of 2013, the first consideration was the ethical protocol of the study by Shrestha and Dunn (37), which it was approved by the Ethics Committee of the College of Medicine, University of Misan (Ref No.2025.02.16.42). Informed consent was obtained, and consent was kept on file for all study participants before sampling.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgment

We appreciate the participation of the employees of Baghdad Medical City Teaching Hospital and their hard work and support throughout the research study. No conflicts of interest declared.

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