Kirkuk Journal of Science Vol. 20, Iss. 3, p. 66-77, 2025 doi:10.32894/kujss.2025.162282.1225

Effect of Bacterial Infection on TLR4 Concentration and Its Gene Polymorphism in Acute Leukemia Patients in Erbil Province/ Kurdistan.

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Article Information

Article Type:
Research Article

Keywords:
Acute leukemia; Bacterial infection; TLR4 level and Gene polymorphism.

History:
Received: 3 July 2025
Reviced: 13 August 2025
Accepted: 13 August 2025
Published: 30 September 2025

Citation: Sara rostam omar hussien and Suhaila nafee darogha, EEffect of Bacterial Infection on TLR4 Concentration and Its Gene Polymorphism in Acute Leukemia Patients in Erbil Province/Kurdistan., Kirkuk Journal of Science, 20(3), p.66-77, 2025, https://doi.org/10.32894/kujss.2025.162282.1225

Abstract

Toll-like receptors (TLRs) are essential to initiate an immune response to ward an infection. The current study sought to examine the correlation between Single Nucleotide Polymorphisms (SNPs) and the expression of the TLR4 gene (896A/G) concerning the risk and prognosis of acute leukemia in Erbil Province, as well as to evaluate the influence of bacterial infections on serum TLR4 levels and associated gene polymorphisms in patients diagnosed with acute leukemia. Serum TLR4 levels were markedly elevated in patients with acute leukemia, particularly in those with ALL. Post-treatment study revealed a substantial reduction in TLR4 levels in AML; however, the decrease was not statistically significant in ALL. Additionally, bacterial infection before and after chemotherapy exhibited no significant correlation with TLR4 expression. Gene polymorphism analysis indicated no significant connection between the TLR4 (A/G) genotype allele and leukemia risk. Serum levels of TLR4 were elevated in acute leukemia patients, particularly in ALL, and diminished following treatment in AML. The infection state exhibited negligible impact, and no substantial correlation was identified between TLR4, genotypes, and disease risk, but the A allele may merit additional investigation.

1. Introduction:

Leukemias are a class of hematologic diseases that originate from the circulatory system and are linked to malignant neoplasms. It is typified by unchecked leukocyte growth and proliferation [1]. Leukemia can be either acute or chronic, and it is referred to as myelogenous leukemia if the impacted cells are of the granulocyte or monocyte lineage and lymphoblastic leukemia if they are of the lymphocyte lineage [2], [3].

The malignancy of B or T lymphoblasts is known as acute lymphocytic leukemia (ALL) [4] is typified by the unchecked growth of aberrant, immature lymphocytes and their progenitors, which eventually results in the replacement of bone marrow components and other lymphoid organs, giving rise to the typical disease pattern associated with ALL [5]. Complex pathogenicity, which results from the accumulation of genetic defects, is a characteristic of acute myeloid leukemia (AML), an aggressive malignant hematologic neoplasm with a prevalence that is rising with age [6]. Leukemia patients are susceptible to infections as a result of both the illness and the treatment. The main way leukemia suppresses the immune system is by causing neutropenia, which is a major risk factor for infections. Long-term neutropenia brought on by induction chemotherapy for acute leukemia is linked to a significant

risk of infectious consequences, with bacterial pneumonia, sepsis, and fungal infections being the most common [7],[8]. The three primary domains of integral membrane type I glycoproteins, known as Toll-Like Receptors (TLRs), are the extracellular, transmembrane, and cytoplasmic domains. To eradicate harmful microbes, they directly contribute to the regulation of inflammatory responses and the initiation of innate or adaptive immune responses [9]. TLRs are present on the surface of both immune and tumor cells, and their abnormal expression allows cancer cells to evade the immune system while simultaneously promoting their growth, angiogenesis, tumor invasion, and provision of a suppressive microenvironment, the degree to which these receptors are implicated in hematological malignancies varies. The first human TLR to be discovered, TLR4, has recently been proposed as having potential benefits for acute leukemia [10, 11].

The TLR4 gene, which codes for 839 amino acid residues and has three exons, is determined to be on chromosome 9 (9q33.1). The TLR4 gene's coding section, exon 3, contains a missense polymorphism called TLR4 (rs4986790), where an A-G substitution takes place at position 299 of the gene [12, 13].

A variety of infectious diseases and cancers, including hematological malignancies, have been linked to genetic polymorphisms in TLRs, particularly the TLR4 gene. Genetic variations in the TLR4 gene may also affect intracellular signaling in mononuclear cells, which can set off immunological reactions.

Numerous studies investigate the relationship between TLR4 SNPs and cancer risk. Since TLRs are expressed on more primitive hematopoietic stem and progenitor cells, several SNPs of TLRs can impact the genetic susceptibility to hematologic malignancies, including leukemia [14]. The two most common SNPs of the TLR4 gene, rs4986790 and rs4986791, are linked to infection susceptibility and neutrophil physiopathology [15]. This study aimed to evaluate the serum level of TLR4 in acute leukemia patients before and after chemotherapy associated with bacterial infection and single-nucleotide polymorphism variant of the TLR4 gene (rs4986790) and the risk of ALL and AML.

2. Materials and Methods:

2.1 Study Participants:

This research includes a case-control study and the samples collected from 75 newly diagnosed AL which visited the Nanakali hospital for cancer and blood diseases, and 30 healthy controls from August 1, 2024, to March 30, 2024. There were two groups in the study: According to the FAB classification, which took into account clinical symptoms, peripheral blood counts, peripheral blood smear examinations, bone marrow aspiration, and flow cytometry, the control group comprised 30 healthy individuals and 76 newly diagnosed acute leukemia patients (41 ALL and 35 AML).

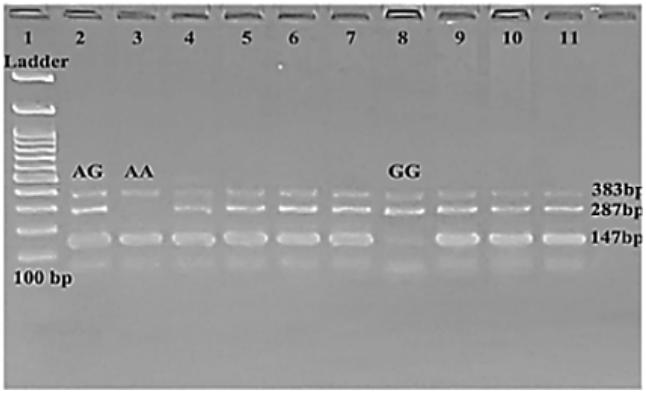

Figure 1. Agarose gel electrophoresis for the TLR4 (896A/G) SNPs.

2.2 Blood Sample Collection:

Three milliliters of peripheral blood samples were taken from healthy groups and new cases of acute leukemia (ALL and AML patients), both before and after chemotherapy. The samples were then put in a gel tube to extract the serum. After centrifuging the tubes for ten minutes at 3,000 rpm, the serum was transferred to a 1.5 μl Eppendorf tube and kept at -70°C until the cytokine level was determined. In order to extract DNA from whole blood or mononuclear cells, roughly 2 mL of the blood samples was also put in EDTA tubes (Becton, China). The tubes were then kept at -70°C until genomic extraction was accomplished.

2.3 Serum TLR4 Measurement using ELISA:

A commercial ELISA kit (Sunlong Biotech Co., China) was used to measure the serum TLR4 concentration after the frozen serum had thawed at room temperature, and the Cloud Clone Corp kit was utilized by the guidelines provided by the manufacturer. The concentration of serum TLR4 was measured in pg/mL. Analysis of the TLR4 ELISA kit (Sunlog, china), was carried out at the Biotechnology Laboratory, Department of Biology, College of Education, Salahaddin University-Erbil.

2.4 DNA Isolation and Genotyping of TLR4 (896A/G) SNPs:

The frozen blood in an EDTA tube was allowed to defrost at room temperature before the AddBio genomic DNA extraction kit (Korea) was used in accordance with the manufacturer's instructions to extract the DNA. Using a nano-drop (Biometrics, One Drop TOUCH Pro/Lite Micro-Volume Spectrophotometer, Wilmington, USA), DNA concentration and purity were found by measuring absorbance at 260 and 280 nm, The concentrations of extracted DNA were varied from 10 to 97 μL. The purity of all the genomic DNA samples was found to be between 1.7 and 1.9.

Table 1. Primer sequences used in T-ARMS-PCR genotyping detection and interpretation.[16]

Gene polymorphism	Primer name	Primer sequence	Allele	Amplicon size (bp)	Product size (bp)	Genoty pe
TLR4 (A/G)	FO	TGAACCCTATGAACTTTATCC		383	NH: 147, 383	AA
	RO	GTTAACTAATTCTAAATGTTG CCATC		383	HE: 147, 287, 383	AG
	FI	GCATACTTAGACTACTACCTCGATGA	A	147	MH: 287, 383	GG
	RI	CAAACAATTAAATAAGTCAATAATAC	G	287		
NH: Normal homozygote; HE: Heterozygote; MH: Mutant homozygote						

The TETRA-ARMS PCR assay was employed to genotype the A and G alleles of the TLR4 gene at position 896. Table 1 lists the primers used in this investigation for TLR4 (896A/G) genotyping were developed according to the procedure defined by the author [17]. Their sequences are given in Table 1 of the distribution. The design focused on the TLR4 SNP (rs4986790) region utilizing the TETRA-ARMS PCR technique. Primer sequences were chosen to provide allele-specific amplification with appropriate melting temperatures and minimal secondary structure formation, adhering to recognized T-ARMS PCR primer design principles. they were adapted from the published study by Alsadawi et al. 2× Prime Taq Premix (genet Bio, Korea: product code 35001) was used to carry out the amplification procedure and the PCR reaction condition for the T-ARMS technique. The optimized PCR reactions were conducted using a 7.0 μl DNA template with a concentration of 10-50 ng/μl, 1.0 μl of each primer, 12.5 μl of 2× Red Master mix (AMPLIQON, Denmark), and 1.5μl of nuclease-free water, resulting in a final volume of 25 μl. The reaction was amplified by using a PCR thermal cycler (Alpha, UK) and the PCR program, which started with a heating temperature of 95 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 35 s at 51 °C, and 45 s at 72 C. Final extension of 72 °C for 10 min.Table 1.

2.5 Urine Culture:

Homogenized urine specimens were inoculated on Blood agar and MacConkey agar medium after midstream urine samples from ALL and AML patients were aseptically collected into a sterile container both before and after chemotherapy. After that, the culture media were incubated at 37°C for 24 hours. Bacterial-growth-free specimens were regarded as negative. Using the VITEK 2 automated microbiology system analyzer, Gram-positive and Gram-negative bacteria were identified.

2.6 Statistical Analysis:

A version of Graph-Pad Prism 9.0 was utilized for all statistical studies. To ascertain the association between genotypes and acute leukemia, ROC curves, estimates of the relative risk (RR), odds ratio (OR), and 95% confidence intervals (CI) were made. Hardy-Weinberg equilibrium (HWE) was used to assess differences in the distribution of the diplotypes between the acute leukemia and healthy control groups. A p-value of less than and equal 0.05 was considered statistically significant.

3. Results:

3.1 TLR4 Level Measurement:

Following statistical analysis, the TLR4e in acute leukemia patients' blood was substantially greater (1390 ± 73.11 pg/mL) than in the healthy group's serum (984.7 ± 34.80 pg/mL) (P = 0.0001), as shown in the ROC curve **??** (A and B). According to the results of the analysis of the ROC curve, the AUC for patients with acute leukemia was 0.767, with a P-value of 0.0001; the cutoff value of TLR4 for predicting severity was 968.3 pg/mL. Figure (1B).

The result shows that the Mean±SEM of TLR4 levels in the serum of ALL patients was significantly elevated compared to the healthy group, with a P value of ¡ 0.0001 (1530 ± 106.0 pg/mL versus 948.7 ± 34.80 pg/mL, respectively) Figure (3A). In contrast, patients with AML also exhibited higher serum levels of TLR4, although this difference was not statistically significant compared to the healthy group (1225 ± 92.90 pg/mL). ROC analysis showed that the increase in serum TLR4 levels in ALL patientsle AUC of 0.815 (p-value = 0.00(p-valuea cut-off value of 1025 pg / ml for serum TLR4 levels, in patients with AML, the AUC was found to be 0.711 (p-value = 0.001). At a cut-off value of 968.3 pg / ml for serum TLR4 levels, as revealed in Figure (3B and C).

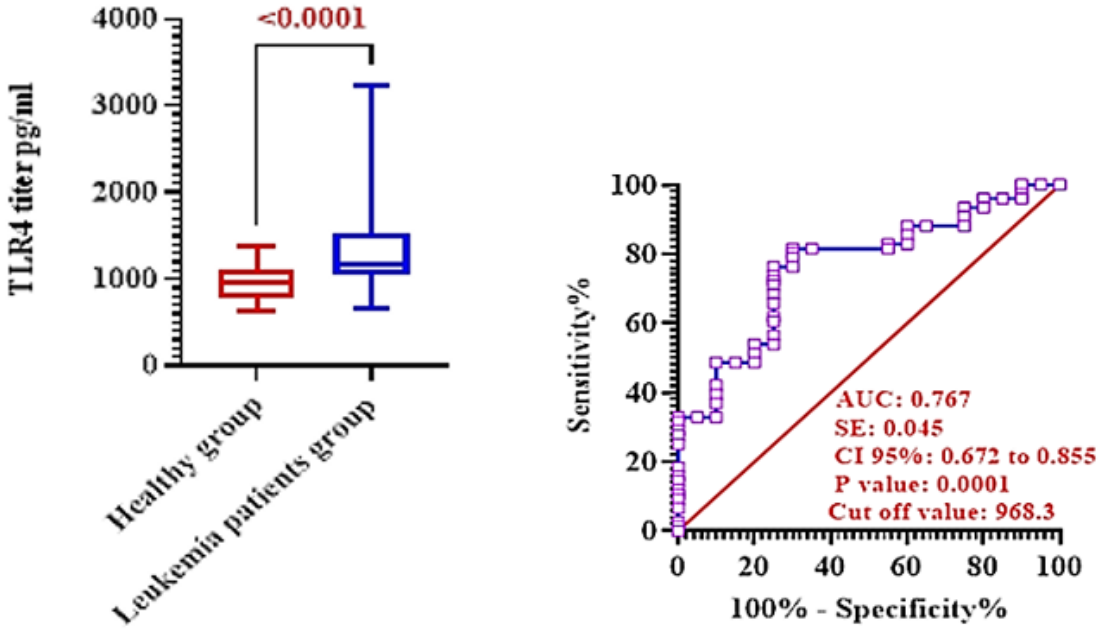

Figure 2. A. Serum level of TLR4 in healthy and leukemia patient's groups.B. ROC curve of TLR 4 and AL patients.

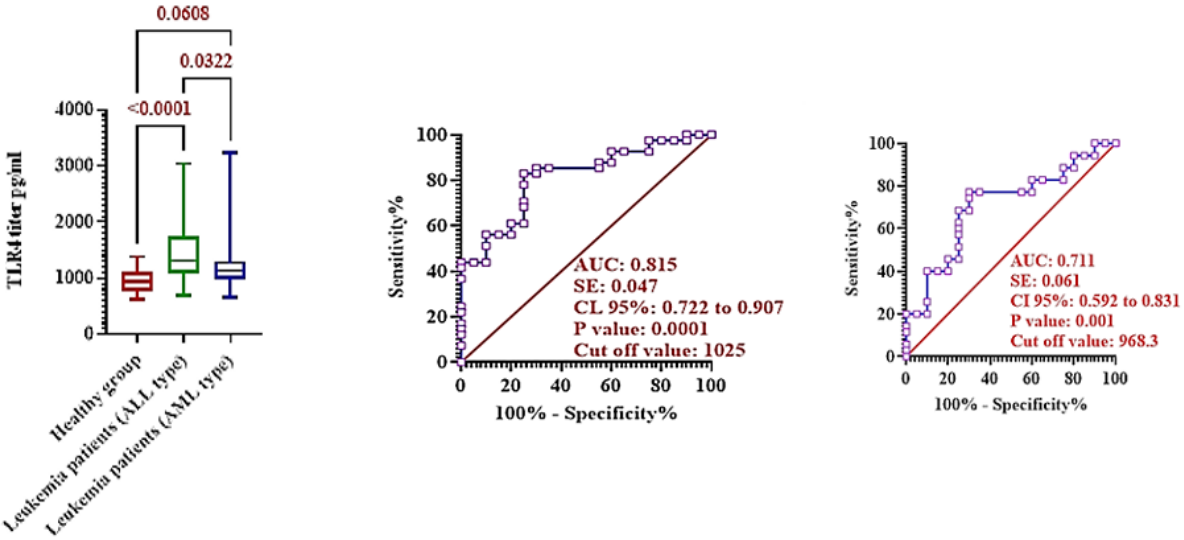

Figure 3. A. Serum level of TLR4 in healthy and leukemia patient's categories. B. ROC curve of TLR 4 and ALL patients. C. ROC curve of TLR4 and AML patients.

The findings indicated that there was no significant difference in serum TLR4 concentrations among the various types of acute leukemia, both prior to and following chemotherapy. In patients with ALL, the concentrations were measured at 1530 ± 106.0 pg/mL and after chemotherapy 1288 ± 119.0 pg/mL, while in AML patients, the values were 1225 ± 92.90 pg/mL and 948.6 ± 21.71 pg/mL, as illustrated in Figure **??** (A and B).

3.2 Isolated Bacteria in Acute Leukemia:

The two pie charts illustrate the frequency of bacterial growth before and after chemotherapy. Before chemotherapy, 53.85% of samples exhibited bacterial growth, while 46.15% showed no growth. Among the samples with bacterial growth, 34.62% were gram-negative bacteria and 19.23% were gram-positive bacteria. After chemotherapy, bacterial growth slightly decreased to 50%, with no growth increasing to 50%. The proportion of gram-negative bacteria declined to 30.77%, while gram-positive bacteria remained at 19.23% Figure 5.

3.3 Association of TLR4 levels with bacterial infection:

The serum TLR4 level in acute leukemia patients exhibited a small, non-significant drop before treatment in the no growth group, from 1030 ± 37.76 pg/mL to 938.3 ± 82.3 pg/mL in ALL patients, and from 1042 ± 68.49 pg/mL to 921.8 ± 31.10 pg/mL in AML patients Figure 5. In patients with gram-positive bacterial isolates, the mean TLR4 level significantly decreased (P= 0.024) from 1487 ± 122.5 pg/mL to 1179 ± 102.6 pg/mL. Conversely, in patients with gram-negative isolates, TLR4 levels exhibited a non-significant reduction from 1834 ± 151.2 pg/mL before chemotherapy to 1568 ± 133.2 pg/mL post-chemotherapy, as illustrated in Figure **??** A. In AML patients infected with gram-positive bacteria, TLR4 levels exhibited a non-significant decrease from 1657 ± 73.61 pg/mL before chemotherapy to 943.2 ± 45.86 pg/mL post-chemotherapy. Conversely, in AML patients infected with gram-negative bacteria, TLR4 levels significantly decreased (P=0.01) from 1200 ± 53.97 pg/mL before chemotherapy to 1007 ± 37.93 pg/mL after chemotherapy, as illustrated in Figure **??** B.

3.4 Molecular Analysis:

Hardy-Weinberg Equilibrium tests found that ALL patients had a significantly different distribution of TLR4-896 genotypes (P=0.005). The observed variation was caused by disparities in the observed and exposed frequencies of AA (wild type), AG(heterozygous), and GG(homozygous), particularly in the heterozygous genotype, which had a frequency of 68.3% versus the predicted frequency of 47.58%. In AML patients, no significant variation from H-W was seen in TLR4-896 genotypes (P= 0.403), as indicated in Table 2.

The prevalence of the rs4986790 polymorphism in TLR4 among 76 acute leukemia patients (41 with ALL and 35 with AML) and 30 healthy individuals was assessed using T-ARMS-PCR, as illustrated in Table 3. The results were as follow; AA; 3(10%), 14(40%) and 2(4.9%) respectively for healthy person, AML and ALL patients, while the distribution of AG; was 18(60%), 28(68.2%) and 18(51.4%) for healthy person, ALL and AML patients respectively. The distribution of GG was as follows: healthy individuals 9 (30%), ALL 11 (26.8%), and AML 3 (8.6%). Furthermore, the frequencies of the A and G alleles were 32 (39.1%) and 50 (60.9%) in all patients, and 46 (65.7%) and 24 (34.3%) in AML patients, respectively. Statistical analysis indicated non-significant differences in the frequencies of TLR4 genotypes AA, AG, and GG among all patients (P= 0.644; P= 0.615; P= 0.795). However, a statistically significant difference was observed in AML patients regarding the distribution of TLR4 genotypes AA and GG (P= 0.010 and P= 0.050), while genotype AG exhibited non-significant differences (P= 0.618). The impairment of the immune system is implicated in the etiology of cancer, with the innate immune system serving a crucial part in the body's defense mechanisms against infections and malignancies, including leukemia [18]. TLR4, a type I transmembrane glycoprotein, possesses an extracellular domain that facilitates the identification of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS). Ligand binding to this structure activates TLR4, initiating a cascade of signal transduction and various inflammatory pathways, primarily through the adaptor molecule myeloid-differentiation protein 88, which activates the nuclear factor kappa beta (NF kB) pathway and promotes the transcription of pro-inflammatory cytokines [19], [20]. Toll-like receptors (TLRs) are expressed by innate immune cells and diverse cancer cells. Numerous studies indicate that TLRs exhibit a dual role in cancer, providing antitumoral effects through robust immune responses, while simultaneously exerting pro-tumoral effects via the secretion of pro-inflammatory cytokines and growth factors that enhance tumor cell proliferation, invasion, and metastasis [21],[22]. Previous study indicates that TLRs are expressed by several hematological malignant cells, including ALL and AML cells. TLR4 and TLR9 may coordinate a signal that enables cancer cells to evade immune responses by enhancing the expression of immunosuppressive cytokines and anti-apoptotic proteins [11]. While TLR signaling may be essential for the development of a proper immune response, evidence suggests a correlation between elevated TLR expression and signaling and hematological malignancies [23]. Previous study indicates that TLRs are expressed by several hematological malignant cells, including ALL and AML cells. TLR4 and TLR9 may coordinate a signal that enables cancer cells to evade immune responses by enhancing the expression of immunosuppressive cytokines and anti-apoptotic proteins

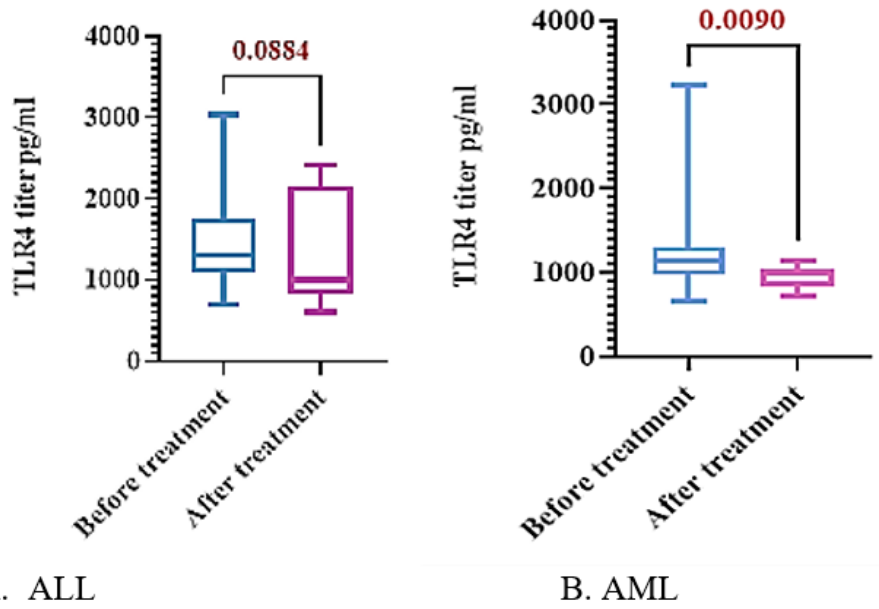

Figure 4. Isolated bacterial frequencies in A. Before chemotherapy, and B. After chemotherapy.

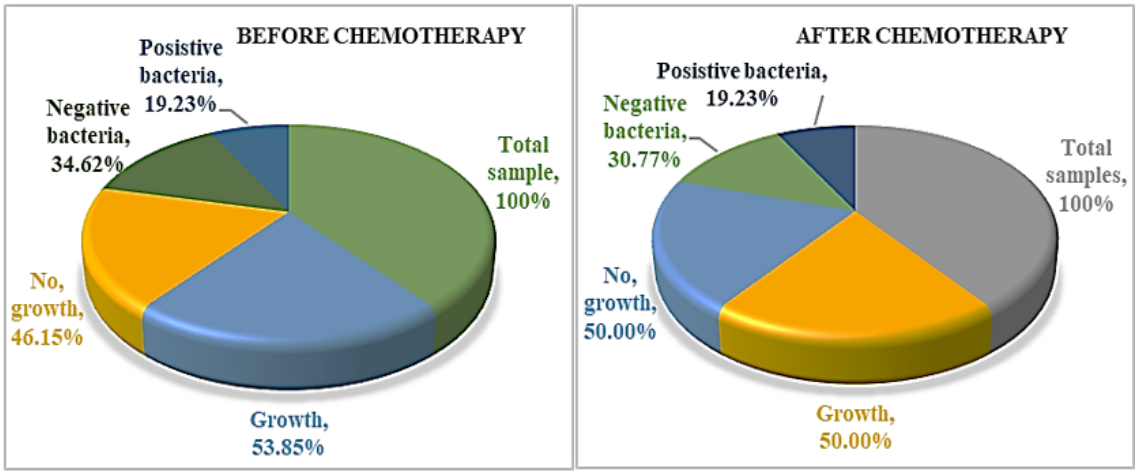

Figure 5. A. Serum level of TLR4 among ALL patients before and after chemotherapy. B. Serum level of TLR4 among AML patients before and after chemotherapy.

Table 2. Hardy-Weinberg equilibrium test for the genotypes and allele distributions of TLR4 A/G in healthy persons and acute leukemia patients.

TLR4 gene at position -896 A/G							
Case Categories		Genotypes			HWE p-value	Alleles	
		AA	AG	GG		A	G
All	Observed	2 (4.9%)	28 (68.3%)	11 (26.8%)	0.005	32 (39.02%)	50 (60.97%)
	Expected	6.24 (15.22%)	19.52 (47.61%)	15.24 (37.17%)		NA	
AML	Observed	14 (40.0%)	18 (51.42%)	3 (8.58%)	0.4037	46 (65.72%)	24 (34.28%)
	Expected	15.12(43.2%)	15.77(45.06%)	4.11(11.74%)		NA	
Healthy Pearson	Observed	3(10%)	18(60%)	9(30%)	0.1709	24(40%)	36(60%)
	Expected	4.8(16%)	14.4(48%)	10.8(36%)		NA	
NA: Not applicable							

[11]. The expression of TLR4 is significantly associated with tumor invasiveness [24]. The present study's findings indicated that acute leukemia patients exhibited substantially higher TLR4 levels than healthy controls (1390 ± 73.11 and 948.7 ± 34.80 pg/mL). The ROC analysis produced an AUC of 0.767 (p = 0.0001), which suggests that TLR4 has moderate diagnostic accuracy and supports its potential as a biomarker for leukemia, particularly at a cut-off value of 963.447 pg/mL. Both types of leukemia (1530 ± 106.0 pg/mL and AML, 1225 ± 92.90 pg/mL) exhibited significantly higher TLR4 levels than healthy controls (948.7 ± 34.80 pg/mL). These results are consistent with global evidence, including a study conducted by [23]. In Mexico, which revealed markedly increased TLR4 expression in leukemia patients' peripheral blood mononuclear cells, underscoring its role in leukemogenesis and immunological dysregulation. In Iraq, research carried out by [25] discovered that ALL patients had higher levels of TLR4 expression than the control group. There is contention regarding TRL4 expression in acute leukemia, as several studies have indicated overexpression [26], while the findings of Pehlivan et al. [27] and Sánchez-Cuaxospa et al. [23] exhibited reduced expression levels relative to normal control The research conducted by Fateh et al. [28], indicates that the expression levels of TLR1/2/4/7/8 are markedly increased in AML patients, implying their potential involvement in cancer start and/or progression. contrary to studies declaring high levels of TLRs in AML patients, also in Iraq, research by [29] Identified elevated inflammatory gene expression in ALL relative to AML, including increased levels of TLR4 and associated cytokines, which they ascribed to variations in immune response profiles between myeloid and lymphoid malignancies. Webb et al. [30] revealed that AML patients had lower TLR4 expression levels than the control group, which resulted in a weaker immune response against cancerous leukemic cells. Understanding how therapy impacts TLR4 expression in distinct leukemia subtypes was crucial to this investigation. AML patients had a statistically significant reduction in TLR4 levels following therapy, from 1288.0 to 134.8 pg/mL (p = 0.023), while ALL patients had a non-significant reduction (p = 0.177). This disparity may reflect underlying biological differences in myeloid and lymphoid leukemias' inflammatory environment and treatment responsiveness. Globally, [4] Whereas microenvironmental resistance often raises TLR4 expression, AML treatment suppresses TLR4 signalling and pro-inflammatory cytokines. An Iraqi study by [31] reported significant post-treatment decreases in TLR4 and IL-6 in AML patients, coinciding with hematologic improvement and remission induction. The documented bacterial growth patterns prior to and during chemotherapy reveal the infection risk linked to acute leukemia and its treatment. Before chemotherapy, 53.85% of patients demonstrated bacterial growth, which marginally declined to 50.0% following treatment. Gram-negative bacteria were the predominant isolates in both instances, underscoring their enduring prevalence in hematological malignancies. Although the reduction lacked statistical significance, it may indicate a slight advantage of chemotherapy-induced immune regulation and adjunctive antimicrobial therapies. Research conducted by [32] underlined that Gram-negative organisms, particularly E. coli and P. aeruginosa, remain the primary causes of bloodstream infections in chemotherapy-treated leukemia patients, with little reduction after initial treatment. A study in Iraq by [33] Similarly, leukaemia patients frequently contracted Gram-negative infections even after receiving induction therapy because of their protracted neutropenia.

In ALL and AML patients, TLR4 levels decreased across bacterial infections after chemotherapy. M. Ramzi et al. [34] discovered no significant connection between microbial infection and TLR4 levels in pediatric ALL patients, suggesting host genetic variables and disease-specific pathways may be

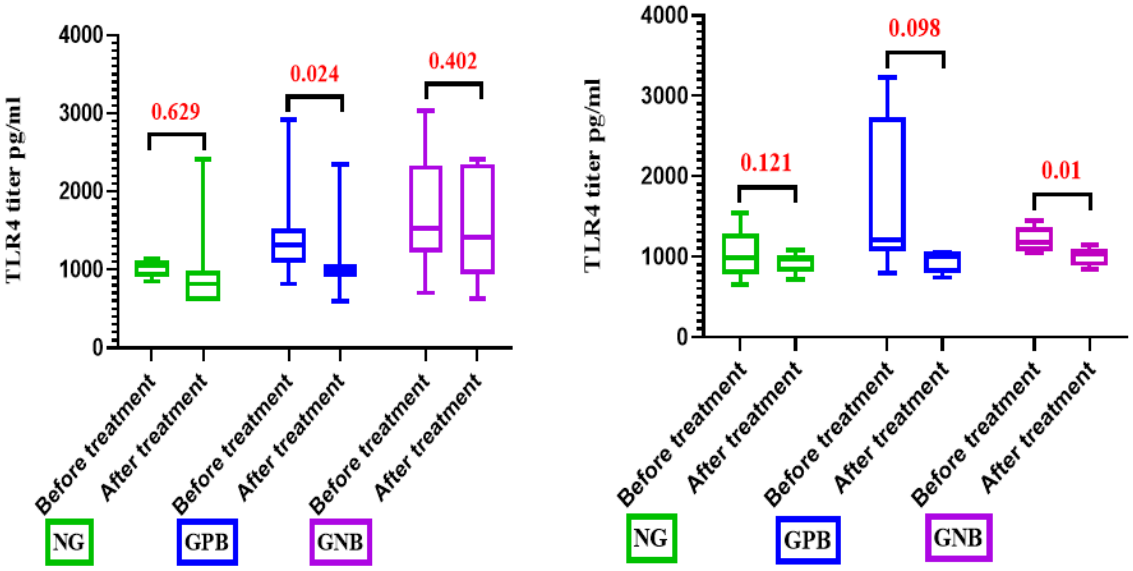

Figure 6. Serum level of TLR4 according to bacterial culture. A. Among leukemia patients ALL type before and after chemotherapy. B. Among leukemia patients AML type before and after chemotherapy.(NG; no, growth, GPB: Gram-positive bacteria, GNB: Gram-negative bacteria).

more important. International research by [35] underlined that TLR4-mediated inflammatory responses in AML are sensitive to cytotoxic treatment and microbial exposure, notably Gram-negative endotoxins, which may initially increase TLR4 but decrease with successful treatment. An investigation conducted in Iraq by[36]. similarly, TLR4 levels were elevated at baseline in AML patients with Gram-negative bloodstream infections, but they returned to normal following chemotherapy and antibiotics.

Analysis of TLR4 (A/G) gene polymorphisms revealed that genotype distributions among ALL, AML, and healthy control groups conformed to HWE, with the AG genotype emerging as the most prevalent across all groups and the G allele appearing more frequently in ALL and the A allele in AML. These findings suggest that there is no significant alteration in the frequency of TLR4 polymorphisms in leukemia patients compared to healthy individuals, indicating that this genetic variant may not play a major role in leukemogenesis in the studied population. Our findings agreed with those reported by Aref et al. [37], who recoded a nonsignificant difference between 120 AML patients and 100 controls regarding genetic variations in the TLR4 gene (rs 4986791) in a cohort of Egyptian patients Egypt and reported no significant differences in genotype or alleles frequency between patients and healthy controls regarding TLR2 rs5743708, TLR4 rs4986790, and rs4986791 polymorphisms. Conversely, our findings disagreed with those of Banescu et al. [6], they found that TLR4 variant genotypes (rs4986791) were related with AML risk in a large Eastern European cohort of 511 AML patients and 503 healthy controls from Romania. As TLR4 is expressed in lymphocytes and regulates B-cell development and activation, genetic polymorphisms may contribute to leukemia etiology and pathophysiology [1]. Few studies have linked TLR4 SNPs to hematological malignancies. A meta-analysis of 55 publications found that TLR4 gene polymorphisms rs4986791 and rs11536889 may be genetic risk factors for cancer, whereas rs4986790 is not [38]. In a study done by Delkhah et al [15], the allelic frequencies of the rs4986790 and rs4986791 TLR4 polymorphisms were identified using PCR-RFLP and ARMS-PCR, respectively, in 5.8

4. Conclusions :

The research highlights the vital function of TLR4 in the immunopathology of acute leukaemia. Elevated blood TLR4 levels were observed in both ALL and AML patients relative to healthy controls, with a more pronounced expression in ALL. A post-treatment investigation revealed a significant decrease in TLR4 levels in AML, although no such alteration was noted in ALL, suggesting divergent immune response mechanisms. Despite a notable incidence of bacterial infections, particularly gram-negative pathogens, no significant association was observed between infection status and TLR4 levels. The genetic analysis of TLR4 (A/G) polymorphisms revealed no significant differences between patients and controls, indicating a negligible impact on leukemogenesis in the studied group.

Funding: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Table 3. The distribution of genotypes and alleles frequency of TLR4 (rs4986790) SNPs among acute leukemia patients and the controls.

Acute Leukemia categories	TLR4 (A/G)	Acute Leukemia patients′ frequency	Healthy person frequency	Relative Risk (RR)	Etiology or Preventive Fraction	Exact Fishers Probability(P)	95% Confidence Intervals (CI)
ALL	Genotype						
	AA	2(4.9%)	3(10%)	0.46	0.05	0.644	0.07 – 2.87
	AG	28(68.2%)	18(60%)	1.44	0.20	0.615	0.55 – 3.78
	GG	11(26.8%)	9(30%)	0.86	0.04	0.795	0.31 – 2.39
	Allele						
	A allele	32(39.1%)	24(40%)	0.96	0.01	1.000	0.49 – 1.89
	G allele	50(60.9%)	36(60%)	2.34	0.035	0.017	1.19 – 4.61
AML	Genotype						
	AA	14(40%)	3(10%)	6.00	0.33	0.010	1.56 – 23.12
	AG	18(51.4%)	18(60%)	0.71	0.17	0.618	0.27 – 1.86
	GG	3(8.6%)	9(30%)	0.22	0.23	0.051	0.05 – 0.88
	Allele						
	A allele	46(65.7%)	24	2.87	0.42	0.005	1.42 – 5.86
	G allele	24(34.3%)	36	0.35	0.39	0.005	0.17 – 0.71

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations:

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: Ethical approval for this study was obtained from the Scientific Committee of Salahaddin University (Approval Code: SU2025HREC), granted on 7 August 2025.

Author contributions: Sara rostam omar hussien: conceptualization; data curation; methodology; writing—original draft; Visualization; writing—review editing. Professor Dr. suhaila nafee darogha: conceptualization; formal analysis; investigation; methodology; project administration; supervision; writing—review editing.

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تأثير العدوى البكتيرية على مستوى *TLR*4 وتعدد أشكاله الجينية لدى مرضى سرطان الدم الحاد في محافظة أربيل / كردستان

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الخلاصة

تركز هذه الدراسة على معادلات فريدولم التكامليةالتفاضلية، والتي تُستخدم بشكل متكرر في مجالات المستقبلات الشبيهة بالتول (*TLRs*) ضرورية لبدء استجابة مناعية للعدوى. سعت الدراسة الحالية إلى فحص العلاقة بين تعدد الأشكال النوكليوتيدية المفردة (*SNPs*) وتعبير جين *TLR*4(896*A*/*G*) فيما يتعلق بخطر وتنبؤ اللوكيميا الحادة في محافظة أربيل، وكذلك تقييم تأثير العدوى البكتيرية على مستويات *TLR*4 في المصل وتعدد الأشكال الجينية المرتبطة بها لدى المرضى الذين تم تشخيصهم باللوكيميا الحادة. تم تسجيل ما مجموعه 76 مريضًا بسرطان الدم من أغسطس 2024 إلى مارس 2025 في مستشفى ناناكالي، أربيل. تم الحصول على البيانات السريرية والاجتماعية الديموغرافية باستخدام استبيان منظم، وتم تقييم مستويات *TLR*4 في المصل عبر اختبار *ELISA* الساندويتش (اختبار الامتصاص المناعي المرتبط بالإنزيم). تم تحديد البكتيريا باستخدام تحليل البول (يدويًا و *VITEK*2)، وتم تقييم تعدد أشكال جين *TLR*4 باستخدام *ARMS* − *PCR* . كانت مستويات *TLR*4 في المصل مرتفعة بشكل ملحوظ لدى المرضى المصابين بسرطان الدم الحاد، وخاصة أولئك المصابين بـ *ALL* . أظهرت دراسة ما بعد العلاج انخفاضًا كبيرًا في مستويات *TLR*4 في *AML* ، ومع ذلك لم يكن الانخفاض ذا دلالة إحصائية في *ALL* . بالإضافة إلى ذلك، لم تُظهر العدوى البكتيرية قبل وبعد العلاج الكيميائي أي ارتباط كبير بتعبير *TLR*4 . أظهرت تحليل تعدد الأشكال الجينية عدم وجود ارتباط كبير بين أليل النمط الجيني *TLR*4(*A*/*G*) وخطر الإصابة بسرطان الدم. كانت مستويات السيروم من *TLR*4 مرتفعة لدى مرضى اللوكيميا الحادة، وخاصة في *ALL* ، وانخفضت بعد العلاج في *AML* . حالة العدوى أ *TLR*4 ، الأنماط الجينية، وخطر المرض، ولكن قد يستحق الأليل *A* مزيدًا من التحقيق.

الكلمات الدالة : سرطان الدم الحاد، العدوى البكتيرية، مستوى *TLR*4 وتعدد أشكال الجينات.

التمويل: لم يتلق المؤلفون أي دعم مالي للبحث أو التأليف أو نشر هذه المقالة.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات: تضارب المصالح: يعلن المؤلفون عدم وجود أي تضارب في المصالح.

الموافقة الأخلاقية: لم يتضمن هذا البحث أي تجارب على البشر أو على الحيوانات، بالتالي لم يكن من الضروري الحصول على موافقة أخلاقية.

مساهمات المؤلفين: سارة رستم عمر حسين: صياغة المفاهيم ؛ معالجة البيانات؛ المنهجية؛ الكتابة ـ المسودة الأصلية؛ التصور؛ الكتابة ـ المراجعة والتحرير. الأستاذة الدكتورة سهيلة نافع داروغا: صياغة المفاهيم ؛ التحليل الشكلي؛ التحقيق؛ المنهجية؛ إدارة المشاريع؛ الإشراف؛ الكتابة ـ المراجعة والتحرير.