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Journal of King Saud University – Science

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## Correspondence

## Association between Toll-like receptor 4 polymorphism and Acute Lymphoblastic Leukemia susceptibility in Saudi Arabian patients

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## ARTICLE INFO

## Article history:

Received 24 October 2021

Revised 27 February 2022

Accepted 16 March 2022

Available online 21 March 2022

## Keywords:

Acute lymphoblastic leukemia

Polymorphism

Gene expression

Toll like receptor

Saudi population

## ABSTRACT

**Objective:** Toll-like receptors (TLRs) play a critical role in initiating an immune response against infections. The present study aimed to investigate the relationship between Single Nucleotide Polymorphisms (SNP) and the expression of TLR4 gene with the risk and prognosis of acute lymphoblastic leukemia (ALL) in the Saudi population.

**Methods:** SNPs at rs4986790 (A/G) rs1927906 (C/T) and rs7856729 (G/T) were genotyped in 265 subjects, including 150 patients and 115 community-matched healthy controls from a Saudi ethnic population. Using quantitative reverse transcriptase PCR, the mRNA expression of TLR4 was compared between the two subgroup.

**Results:** Single nucleotide polymorphisms of the TLR4 in the 3' UTR region and the fourth exon, were found to be associated with the risk of ALL. Individuals carrying the heterozygous genotype for the rs4986790 AG are significantly decreased ALL risk (OR: 0.313; 95% CI: 0.150–0.655;  $P = 0.002$ ). Furthermore, the CT genotype of the rs1927906 was significantly associated with the protection against ALL (OR: 0.305; 95% CI: 0.167–0.557;  $P = 0.00007$ ). Moreover, the mRNA expression of TLR4 was significantly higher in patients with ALL than in healthy individuals ( $P < 0.001$ ).

**Conclusion:** This is the first study focusing on the TLR4 gene variants in leukemia patients in the Saudi population. These results suggested that TLR4 rs4986790 (AG) and rs1927906 (CT) polymorphisms may play an important protective role against ALL. In addition, the increased expression of the TLR4 gene in leukemic samples could serve as a novel potential prognostic factor for this receptor, especially in ALL cases.

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## 1. Introduction

Cancer is known to be a major public health issue. Between 1990 and 2018, Leukemia cases have shown a significant increase from 297,000 to 437,033 globally (Bawazir et al., 2019). The Gulf Cooperation Council (GCC) reports on cancers ranked leukemia as

the 4th most common cancer among GCC nationals, it was also reported by the Saudi Cancer Registry that leukemia was the 5th commonly spread type of cancer within both genders in Saudi Arabia (GCCCP, 2011). It is worth mentioning that the total incidence of childhood leukemia in Saudi Arabia has jumped from 1.58/100,000 in 2001 to 2.35/100,000 in 2014 (Jastaniah et al., 2020).

Leukemias are a group of hematologic disorders associated with malignant neoplasm that arises from the hematopoietic origin. It's characterized by uncontrolled proliferation and development of leukocytes (Sampaio et al., 2021). Leukemia can be classified as myeloid or lymphoid based on the expression of several antigens and markers and according to the predominant type of leukemic blast cells (Arber et al., 2016), if the affected cells are from granulocytes or monocytes lineage, leukemia will be known as myeloge-

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Peer review under responsibility of King Saud University.



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nous leukemia, and if they are from lymphocytes lineage, leukemia will be known as lymphoblastic leukemia (Shafique and Tehsin, 2018). Furthermore, leukemia can be more classified according to the stage of maturation arrest and the degree of cell differentiation into either acute (fast-growing) or chronic (slower-growing) with more mature and functional cells (Arber et al., 2016).

Acute Lymphoblastic Leukemias (ALL) is characterized by an uncontrolled spread of hematopoietic precursor cells of the lymphoid lineage within the bone marrow niche in most cases, and peripheral blood (Witkowski et al., 2019). Although, it has a bimodal age distribution, first early peak between 2 and 5 years of age, and a second steady increase at about age 50 years (Zhang et al., 2019).

Studies showed that multiple hematological malignancies and lymphoid precursor cells from different subtypes of acute leukemia express distinct and heterogeneous profiles of Toll-like receptors (TLRs) transcripts and could respond to stimulation by TLR agonists. Upregulated TLR expression influence the bone marrow microenvironment in the very early fate decisions of the hematopoietic development and regulation (Dorantes-Acosta et al., 2013; Pandey et al., 2015).

Understanding the cellular and molecular foundation and genetic risk factors involved in leukemia etiology is essential. Several regulatory mechanisms exist that may lead to the onset of the disease and implicate in the makeup of the microenvironment of the tumor and the immune status in cancer. Consequently, uncontrolled activation of innate immunity and sustained cytokine production for a more extended period could serve as a platform for developing human cancers since TLRs and inflammasomes are the fundamental sensing molecules of innate immunity to induce specific pro-inflammatory responses (Keshavarz et al., 2021).

TLRs are a family of pattern-recognition receptors (PRRs) that identify and detect conserved sequences and structural motifs in a wide range of pathogens or PAMPs (pathogen-associated molecular patterns), along with damaged tissue or DAMPs (damage-associated molecular pattern) in addition to cancer debris, thus, provokes a host defense response (Ureña-Peralta et al., 2020). A candidate gene that might be associated with the risk of developing ALL is Toll-like receptor 4 (TLR4).

TLR4 is one of the major initiators of the innate immune response which promotes adaptive immunity. TLR4 is 1 of 11 known mammalian membrane-spanning receptors, acting as “bridging molecules” between innate and adaptive immunity and their genes have been reported to be polymorphic, especially due to their location in the ligand recognition area of the receptor. Recently, many research findings approved the functions and molecular mechanisms of TLRs in cancers (El-Omar et al., 2008; Farrugia and Baron, 2017).

Genetic variations caused by Single Nucleotide Polymorphisms (SNPs) in TLRs contribute to the alterations in the immune system, and those variations have been determined in different types of tumors, and their functionality goes far beyond the innate immune system, particularly the association of TLR4 polymorphism with cancer risk. Additionally, several studies examine the association between overall cancer risk or cancer-specific risk and SNPs of TLR4. Multiple SNPs of TLRs can affect the genetic susceptibility to hematologic malignancies, especially leukemia and non-Hodgkin lymphoma because TLRs are expressed on more primitive hematopoietic stem and progenitor cells (HSPCs). Considerable evidence has indicated that the deregulation of the innate immune system receptors may increase the risk for leukemia. Alterations in the expression of TLR4 were found in leukemic cells before and after chemotherapy induction (Ramzi et al., 2018; Paracatu and Schuettelpelz, 2020). Therefore, TLR4 signaling is essential for tumor cell proliferation and immune response. Understanding such polymorphisms and their associated pathologies would provoke a

novel therapeutic approach concentrating on halting the abnormal proliferative activity or developing some mediators that can override the signals to block abnormal hematopoietic differentiation. Accordingly, we thought that functional polymorphisms that occur on the TLR4 gene might have a significant role in the inflammation mechanism and might trigger cancerogenesis through disruption of inflammation.

The present study was conducted to explore the relationship between rs4986790 A/G, rs1927906 C/T and rs7856729 G/T variants of TLR4 polymorphisms with ALL risks in a Saudi population and to investigate new genetic biomarkers for ALL by analyzing the TLR4 polymorphisms at the fourth exon and 3' UTR region.

## 2. Materials and methods

### 2.1. Ethical consideration

All procedures were in accordance with the Helsinki protocol and approved by the medical ethics committee in King Khalid University Hospital and the ethics committee of King Saud University, Riyadh, Saudi Arabia. (Ref. No. 20/0525/IRB).

### 2.2. Inclusion criteria for sample selection

Human whole blood samples of 265 individuals were collected for this study. The ALL samples comprised blood samples isolated from 150 Saudi patients diagnosed with Acute Lymphoblastic Leukemia and with no other known pathologies, hematological disorders, or previous cancer. In addition, 115 unrelated healthy individuals without any clinical signs of cancer or other diseases of both genders (female and male) served as controls.

The mean age of the study population was  $22.45 \pm 20.27$  years for the patients with ALL and  $18.68 \pm 15.53$  years for the healthy controls. No significant inter-group differences were recorded for age ( $P = 0.09$ ).

### 2.3. DNA extraction

Two milliliters of blood samples were collected by venipuncture from each individual and then stored in ethylenediaminetetraacetic acid (EDTA)-containing tubes and stored at  $-20^\circ\text{C}$  before analysis.

Genomic DNA was extracted from blood samples using QIAamp DNA Mini Kit following the recommendation of the manufacturer. DNA purity and concentrations were determined by spectrophotometric measurement of absorbance at 260 and 280 nm (A260/A280 ratio) using Nanodrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

### 2.4. RNA isolation and cDNA synthesis

Total RNA was isolated from 17 patients with newly diagnosed ALL and 17 matched healthy individuals using the QIAamp<sup>®</sup> RNA Blood Mini kit for total RNA purification from human whole blood (QIAGEN). RNA concentration was determined spectrophotometrically, the ratio of absorbances at 260 and 280 nm measured in a Nanodrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used as a parameter to quantify and evaluate the quality of the total RNA extracted. RNA was reversely transcribed into cDNA, following the manufacturer's instructions, the reverse transcription step was carried out using AMV Reverse Transcriptase (Promega, Madison, USA).

2.5. Single nucleotide polymorphisms (SNPs) selection, genotyping, and quality control

The tagging SNPs in TLR4 were selected from The International HapMap website (<https://hapmap.ncbi.nlm.nih.gov>) using genotype data. The SNPs with the frequency of minor alleles greater than 0.05 were selected to predict the possible function. TLR4 polymorphisms were determined by TaqMan allelic discrimination with a TaqMan SNP Genotyping Assay using Real-time PCR.

We genotyped three SNPs in the TLR4 (rs4986790 (Asp299Gly) A/G, assay ID: C\_11722238\_20), (rs1927906 C/T, assay ID: C\_11722136\_10), (rs7856729 G/T, assay ID: C\_43308526\_20). Furthermore, these SNPs are located in the fourth exon and 3' UTR region. The SNPs positions are described in Table 1.

TLR4 polymorphisms were genotyped using the allelic (VIC- and FAM-labeled) discrimination method according to the manufacturer's instructions and as described previously by Livak (1999).

Briefly, forward and reverse primers were designed by the wild-type probe VIC<sup>®</sup> dye (linked to the 5' end of the Allele 1 probe) and the variant probe 6FAM<sup>™</sup> dye (linked to the 5' end of the allele 2 probe) for the sequence of interest. Primers and TaqMan<sup>®</sup> Genotyping Master Mix were purchased from the assays-on-demand service of Applied Biosystems (Thermo Fisher Scientific, Applied Biosystems, USA).

DNA (10–20 ng) from each sample was added to 12.50 µl of TaqMan Genotyping Master Mix (2x) and 1.25 µl of SNP Genotyping Assay mix (2x). Amplification was performed in a final reaction volume of 25 µl.

The following amplification cycle conditions were used: 95 °C for 10 min as initial step, followed by 40 cycles of 95 °C for 15 s, and 40 cycles at 60 °C for 60 s. The PCR conditions were alike for all tested polymorphisms.

Analyses of amplification products and the allelic discrimination were achieved using ViiA<sup>™</sup>7, v.1.1. (Applied Biosystems, USA). A total of 5% of the selected samples were randomly selected for repeat analysis as quality control.

2.6. Quantitative of gene expression by Real-Time PCR

Quantitative real-time PCR was performed to determine the expression level of TLR4 mRNA in blood samples of ALL patients and controls.

Expression levels of TLR4 and the housekeeping gene Glycer-aldehyde 3-phosphate dehydrogenase (GAPDH) (as internal control) genes were assessed using ViiA<sup>™</sup>7, v.1.1. (Applied

Biosystems, USA). The reactions were performed in triplicate, each 20 µl reaction mixture contained 10 µl of SYBR Green PCR Master Mix, 5 µl forward and reverse primers and 2.5 + 2.5 of cDNA template + RNase-free water (Sequences of the primers were shown in Table 2).

Specific PCR conditions were as follows: 95 °C for 5 min followed by 45 cycles of 95 °C for 15 s, 60° C for 1 min, and 72 °C for 30 s, and 72 °C for 5 min.

Relative quantitation of TLR4 and GAPDH mRNA expression was calculated with the comparative threshold cycle method. The general process for analyzing the data from gene expression assays: The amplification plots were viewed, the baseline and threshold values were adjusted to determine the threshold cycles (C<sub>T</sub>) for the amplification curves and the relative expression of TLR4 was calculated using the comparative C<sub>T</sub> method (the 2<sup>-ΔΔC<sub>T</sub></sup> method) (Schmittgen and Livak 2008).

2.7. Statistical analysis

The Hardy-Weinberg equilibrium test was performed for each SNP to detect any deviation in the control samples. Allelic and genotypic frequencies of TLR4 SNPs were compared between patients with ALL and the control group to identify polymorphisms that could associate with the susceptibility to ALL by χ<sup>2</sup> test, Odds ratios (OR) and 95% confidence intervals (CIs) were calculated to evaluate the disease risk. As well, P value < 0.05 was considered statistically significant.

3. Results

Three TLR4 SNPs (rs4986790 A/G, rs1927906 C/T, rs7856729 G/T) were genotyped and assessed for deviation from the Hardy-Weinberg equilibrium (HWE) (P > 0.05). A total of 265 were enrolled in this section, 150 patients with ALL and 115 unrelated individuals as healthy control. The strength of the association between these polymorphisms and risk of ALL cancer was measured by ORs with 95% CIs have shown in Tables 3–5, which summarizes allelic associations between the selected SNPs of the TLR4 in a patient with ALL and healthy individuals.

3.1. Association of TLR4 polymorphisms with ALL

3.1.1. TLR4 (rs4986790 A/G) polymorphism

In terms of the genotype and allele frequencies in rs4986790 A/G polymorphism, there was a remarkable difference between ALL

**Table 1**  
The location and genotype of the selected SNPs included in this study.

SNP	Chromosome	Cytogenetic location	Genotype	Genomic location	Consequence
rs4986790	9	9q33	AA AG GG	117713024	fourth exon
rs1927906			TT TC CC	117717837	3 Prime UTR
rs7856729			GG GT TT	117719578	3 Prime UTR

**Table 2**  
The primer sequences and annealing temperature.

Gene	Forward primer	Reverse primer	Product length	Annealing temperature
TLR4	CCGTTTTATCACGGAGGTGGT	CTGCCTAAATGCCTCAGGGG	135	60
GABDH	AATGGGCAGCCGTTAGGAAA	AAAAGCATCACCCGAGGAG	133	60

**Table 3**  
TLR4 (rs4986790 A/G) genotype frequencies in patients with ALL and controls.

Genotype	ALL patients n = 150		Controls n = 115		Control vs Patients			
	Count	%	Count	%	OR	CI	$\chi^2$	P-value
AA	138	92	90	78	Reference = 1 0.313 NA	0.150–0.655 NA	9.520 –	<b>0.002</b> –
AG	12	8	25	21.7				
GG	0	0	0	0				
<b>Allele</b>								
A	150	0.96	115	0.89	Ref. 0.342	0.168–0.696	9.46	<b>0.0021</b>
G	0	0.04	0	0.11				

Abbreviations; OR, odds ratio; CI, confidence interval;  $\chi^2$ , Chi square; n, number of individuals; Boldfaced values indicate a significant difference at the P > 0.05 level.

**Table 4**  
TLR4 (rs1927906 C/T) genotype frequencies in patients with ALL and controls.

Genotype	ALL patients n = 150		Controls n = 115		Control vs Patients			
	Count	%	Count	%	OR	CI	$\chi^2$	P-value
TT	124	82	72	62.6	Reference = 1 0.305 0.968	0.167–0.557 0.225–4.169	15.74 0.00	<b>0.00007</b> 0.96490
CT	21	14	40	34.7				
CC	5	3	3	1.9				
CT+CC	26	17.3	43	37.4	0.351	0.199–0.619	13.60	<b>0.00023</b>
<b>Allele</b>								
T	145	0.90	112	0.80	Ref. 0.461	0.282–0.754	9.80	<b>0.00175</b>
C	5	0.1	3	0.2				

Abbreviations; OR, odds ratio; CI, confidence interval;  $\chi^2$ , Chi square; n, number of individuals; Boldfaced values indicate a significant difference at the P > 0.05 level.

**Table 5**  
TLR4 (rs7856729 G/T) genotype frequencies in patients with ALL and controls.

Genotype	ALL patients n = 150		Controls n = 115		Control vs Patients			
	Count	%	Count	%	OR	CI	$\chi^2$	P-value
GG	102	68	79	68.6	Reference = 1 1.071 0.387	0.630–1.819 0.034–4.348	0.06 0.63	0.80073 0.42566
GT	47	31	34	29.5				
TT	1	0.6	2	1.7				
GT+TT	48	32	36	31.1	1.033	0.612–1.741	0.01	0.90399
<b>Allele</b>								
G	149	0.84	113	0.83	Ref. 0.986	0.621–1.568	0.00	0.95372
T	1	0.16	2	0.17				

Abbreviations; OR, odds ratio; CI, confidence interval;  $\chi^2$ , Chi square; n, number of individuals; Boldfaced values indicate a significant difference at the P > 0.05 level.

patients and healthy controls, shown in Table 3. The homozygous state AA was detected in 92% of patients and 78% in healthy individuals. Our analysis revealed that subjects with the G allele were more likely to be normal compared with those bearing the A allele. Additionally, out of the three genotypes, only the AG, showed a significant association with a protective effect against ALL (OR: 0.313; 95% CI: 0.150–0.655; P = 0.002). It is worth noting that none of the investigated patients or healthy individuals in this study were a carrier of GG genotype.

### 3.1.2. TLR4 (rs1927906 C/T) polymorphism

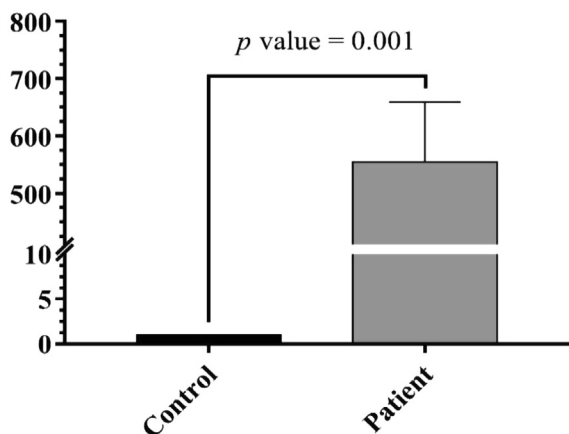
Genotypes of rs1927906 in the wild type homozygous (TT), heterozygous (CT), and homozygous for the variant CC are shown in Table 4.

Our result indicated that the presence of the TLR4 rs1927906 C/T polymorphism in 3' UTR region was associated with decreased cancer risk. Heterozygous CT was decreased significantly among patients with ALL (OR: 0.305; 95% CI: 0.167–0.557; P = 0.00007). Individuals with the CT genotype when considering the recessive model CC genotype in rs1927906 were significantly lower risk of developing ALL.

Furthermore, the recessive genotypes (CT + CC) were also significantly decreased in patient with ALL (OR: 0.351; 95% CI: 0.199–0.619; P = 0.00023). The majority of patients and controls were with homozygous variant genotype (TT). For the C allele, our analysis showed that the allele frequency is significantly decreased the risk of developing ALL, where the T allele frequency was higher in patients with ALL compared to C allele (OR: 0.461; 95% CI: 0.282–0.754; P = 0.00175). The results showed that C allele may be a protective factor against ALL.

### 3.1.3. TLR4 (rs7856729 G/T) polymorphism

Among the three SNPs in TLR4 that were analyzed, the allele and genotype frequencies of rs7856729 G/T polymorphism in the 3' UTR region show no statistically significant difference between healthy individuals and patients with ALL. According to rs7856729 SNP, the P-value was > 0.05 for all genotypes and alleles. Table 5 illustrate the base pairs in the wild type homozygous (GG), heterozygous (GT), and homozygous for the variant (TT). Overall, the majority of patients and controls were with homozygous variant genotype (GG).



**Fig. 1.** Higher Relative expression of TLR4 in the blood samples of ALL patients compared to healthy individuals ( $p < 0.001$ ).

### 3.2. Association of TLR4 relative mRNA expression

To determine whether there was a link between TLR4 expression and ALL, we analyzed TLR4 mRNA expression level in 17 ALL patients and 17 matched healthy individuals using Quantitative RT-PCR. Analysis of the expression data revealed that TLR4 mRNA expression of ALL patients was higher than those of healthy donors. A significant statistical difference was detected between the patients compared to healthy individuals (fold change: 554.402,  $p$  value  $< 0.001$ ) (Fig. 1).

## 4. Discussion

TLRs play an important role in the innate immune system by regulating inflammatory reactions and activating adaptive responses to eliminate harmful pathogens. A large number of SNPs have been discovered in the genes of these receptors. TLR genes have polymorphic nature, so as a consequence, genetic variations in these genes have the ability to affect the pathogenesis of different diseases. Some associations between TLR4 polymorphisms and the susceptibility to infections, inflammation and risk of various cancers have been previously reported (Ding et al., 2017; El-Omar et al., 2008; Ferwerda et al., 2008).

This is the first study investigating whether polymorphisms of TLR4 are associated with ALL development among individuals in the Kingdom of Saudi Arabia. Thus, it would enable the researchers to improve the identification of early diagnostic tools of potential markers.

TLR4 acts in synergy with innate immune cells, and that may contribute to the inflammatory mechanism, which in a genetic perspective could enhance carcinogenesis in some conditions (Martínez-García et al., 2020) since TLR4 is expressed in the lymphocytes and participate in the regulation of B-cell differentiation and activation, consequently, genetic variation of TLR4 gene may contribute to the pathogenesis of leukemia (Rožková et al., 2010; Priyadarshini et al., 2013).

Several previous studies have suggested an influence of polymorphisms in TLR genes encoding factors related to the innate immune response on the etiology of some hematological malignancies subtypes; non-Hodgkin lymphoma, diffuse large cell lymphoma, Hodgkin's lymphoma, acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) and have been reported to be a risk factor (Forrest et al., 2006; Mollaki et al., 2009; Rybka et al., 2016; Ali et al., 2017).

In addition, various studies have been carried out to evaluate the correlation of TLR4 rs4986790 polymorphism with different types of cancer, such as gastric cancer (Zhou et al., 2014), colorectal cancer (Moaz et al., 2020), prostate cancer (Priyadarshini et al., 2013), and lung cancer (Kurt et al., 2016).

Conversely, some studies do not support the role of TLRs genes in cancer risk. For example, Rybka et al. (2016) did not observe any significant relationships between the nonsynonymous TLR4 polymorphisms rs4986790 in AML patients. A meta-analysis study for the association between rs4986790 polymorphism and cancer risk, did not find any strong association between this polymorphism and different types of cancer (Ding et al., 2017). Moreover, the TLR4 Asp299Gly variant was inversely associated with diffuse large cell lymphoma (Forrest et al., 2006) and positively associated with the risk of mucosa-associated lymphoid tissue lymphoma (Nieters et al., 2006), whereas the TLR2 -16933 T>A polymorphism was found to be associated with an increased risk of follicular lymphoma and a decreased risk of CLL (Hellmig et al., 2005).

This study showed that the genotypes AG of the rs4986790 SNP and CT and TT of the rs1927906 SNP are associated with decreased susceptibility to ALL in the Saudi population, thus may cause mild disease outcomes or can develop a balanced inflammatory response, while polymorphism of rs7856729 was found irrelevant to acute leukemia risk. The frequency of the wild allele (A) of the rs4986790 at TLR4 gene in healthy Saudis was 100%; similar to that reported in other populations such as Southern Han Chinese, Han Chinese in Beijing in China, and Japanese in Tokyo. Moreover, the minor allele (G) frequency of the same SNP in healthy Saudis was 0%; is similar to aforementioned populations but different to others including Qatar (0.051), Esan in Nigeria (0.0505) and British (0.0440) (1000 Genomes Project Consortium, 2015).

It has been reported that the substitution of adenine (A) to guanine (G) in the fourth exon in TLR4 rs4986790 may disrupt the extracellular domain of TLR4, consequently, this may reduce signaling via TLR4 which diminished production of inflammatory cytokines (Arbour et al., 2000).

Furthermore, studies have shown that wild allele A is associated with enhanced activation of TLR4 in different inflammatory diseases due to increased levels of inflammatory cytokines like in type 2 diabetes and atherosclerosis (Steinhardt et al., 2010; Kolek et al., 2004).

However, the presence of the AA genotype in rs4986790 might increase the TLR4 activation and susceptibility to ALL since 92% of patients carry this genotype. So, further extensive studies with larger sample size and in other populations should be conducted to confirm this result.

A highly significant difference in TLR4 rs1927906 frequencies was found between the case and control groups. The total recessive, heterozygous genotype and C allele carriers were less susceptible to ALL. Contradictory to our results, concerning other types of cancer or diseases, TLR4 rs1927906 polymorphism is not associated with susceptibility to prostate cancer risk (Lindström et al., 2010) and type 2 diabetes (Kolz et al., 2008).

Generally, the T allele and TT genotype frequencies in this SNP in the healthy participant were 0.80 and 0.626 respectively. This is similar to the data from multiple populations such as in Finland (0.8333) and in Puerto Ricans (0.8317) However, the frequency of the Saudi T allele was higher than that in many populations such as Yoruba in Ibadan, Nigeria (0.4630), Mende in Sierra Leone (0.4941). Additionally, the minor allele (G) frequency of the same SNP in healthy Saudis was 0.2, this is similar to many populations such as: Indian Telugu from the UK (0.2206) and Bangladesh (0.2035) (1000 Genomes Project Consortium, 2015).

No association in our study was found between our TLR4 cohort and the candidate SNP for rs7856729 G/T and ALL. On the other hand, there have been very few publications based on this SNP

ID. TLR4 rs7856729 and rs1554973 were significantly associated with IL-1 $\alpha$  and IL-1 $\beta$ , and IL-10, which involved in the immune response, are correlated with cervical pro-inflammatory cytokine concentrations (Wang et al., 2021). We suggested that the Ethnic differences in genetic susceptibility genes could be a reason for these differences.

Over the recent years, many studies analyzed the role of TLR4 mRNA expression in hematological malignancies and different types of cancer. In our study, overexpression on TLR4 was observed ( $\geq 500$ -fold,  $P < 0.001$ ) in patients with ALL. A possible effect of the studied rs4986790 and rs1927906 SNPs on the upregulation versus downregulation of TLR4 is not excluded. It has been suggested that TLRs high expression in patients might be associated with the induction of different immune mechanisms enabling malignant cells to survive and stimulating carcinogenesis (Rybka et al., 2015). The results of our study are in line with some previous studies concerning the role of TLR4 expression levels in different hematological malignancies and cancers (Ramzi et al., 2018; Bagratuni et al., 2019; Zhao et al., 2019). Therefore, our result may reflect that the high mRNA expressions of TLR4 may contribute to ineffective hematopoiesis and cell proliferation in ALL cases.

## 5. Conclusion

rs4986790 and rs1927906 polymorphism of TLR4 gene could modulate the susceptibility towards ALL disease in the local population of Saudi Arabia. A deeper understanding of the genetic variations of TLR4 will enable us to better identify biomarkers for early detection and prognosis and improve the decision-making procedure of ALL treatments.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

This project was supported by the Researchers Supporting Project number (RSP-2021/35), King Saud University, Riyadh, Saudi Arabia.

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