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Genetic Polymorphism of Toll-Like Receptor 4 Gene (rs4986791) among Adult Patients with Acute Myeloid Leukemia

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Abstract

Background: Toll-like receptor 4 (TLR4) is an essential component of innate cellular immunity. Moreover, TLR4 gene polymorphisms have been proven to be associated with risk to various forms of cancer, including hematological malignancies. Acute myeloid leukemia (AML) is an invasive hematological malignancy. **Objective:** This study aimed to investigate the variability and distribution of a single nucleotide polymorphism (SNP) of the TLR4 gene (rs4986791-Thr399Ile) in AML and to reveal the association of this SNP with the risk of bacterial infection among adult AML patients. **Methods:** A total of 140 adult participants (80 AML patients and 60 healthy controls) were involved in this study. All subjects underwent measurements of baseline hematological parameters. Using real-time PCR, all subjects were investigated for TLR4 (rs4986791) genotyping. **Results:** The homozygous wild-type (CC) variant of TLR4 (rs4986791) was more predominant in adult AML patients (95.00%) and controls (93.33%) than the heterozygous (CT) variant. There was no significant difference in the genotype or allele distribution of TLR4 (rs4986791) between adult AML patients and controls. Likewise, no significant difference in the distribution of TLR4 gene (rs4986791) variants was revealed among AML patients regarding AML different subtypes or acquiring bacterial infection ($P > 0.05$). Moreover, the results of regression analyses revealed no associations between TLR4 (rs4986791) SNP and AML subtypes or bacterial infection in AML patients. **Conclusion:** The homozygous wild-type (CC) genotype of TLR4 (rs4986791) is predominant among adult AML patients and healthy controls. Polymorphism of the TLR4 gene (rs4986791)does not appear to be associated with AML risk, AML different subtypes, and the risk of bacterial infection in the studied AML cohort.

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Keywords: Acute myeloid leukemia; Toll-like receptor 4; Single nucleotide polymorphism; Genotypes; Alleles

1. Introduction

Leukemia is a hematological illness in which the bone marrow abnormally overproduces white blood cells. The excessive production of white blood cells (WBCs) results in a bone marrow accumulation of young WBCs, which in turn causes a decrease in healthy blood cells and inhibits their function [1,2].The hematologic classification system, the French-American-British (FAB) system, relies on leukemia-forming cells to classify this disorder into four types, including acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL) [2,3]. AML is a severe malignant hemato-logic neoplasm that increasesin incidence with age and has a complex pathogenicity caused by the accumulation of genetic abnormalities [4,5]. In adults,AML is the most common type of acute leukemia [5,6]. The global estimate of AML has grown from 79,372 patients in 1990 to be 144,645 cases by 2021 [7]. In Egypt, leukemia constitutes 10% of all cancers, with AML constituting 16.9% [8,9]. In patients with AML, many of the white blood cells may appear abnormally as myoblasts (blasts), which are very early forms of blood-forming cells with abnormal existence in blood. These cells do not function properly like normal mature white blood cells [2,10].

The FAB system classified AML into eight subtypes, starting from M0 to M7, depending on the kind of cells from which the leukemia arises [2,10,11]. M0 refers to undifferentiated acute myeloblastic leukemia, M1 refers to acute myeloblastic leukemia with minimal maturation, and M2 refers to acute myeloblastic leukemia with maturation. Moreover, M3 represents acute promyelocytic leukemia. Furthermore, M4 refers to acute myelomonocytic leukemia, and M5 refers to acute monocytic leukemia. Also, M6 represents acute erythroid leukemia, and M7 refers to acute megakaryoblastic leukemia [10,11]. AML subtypes from M0 to M5 all originate in immature forms of white blood cells, including myeloblasts, promyelocytes, granulocytes, monoblasts, and monocytes. On the other hand, the AML M6 subtype originates in very immature forms of red blood cells (RBCs), and the AML M7 subtype originates in megakaryoblasts, which are immature forms of platelet-forming cells [10,11].

Genetic factors are a crucial key player in AML development and occurrence. Therefore, they are employed in the stratification of disease risk [5,12,13,14]. It is revealed that immune system impairments are the main observable characteristics involved in the pathology of cancer [5,15]. Indeed, the innate immunity not only has an important role in the defense mechanisms against several infections but also fights malignancies, including leukemia. Toll-like receptors (TLRs) are proteins that play a chief role in innate immunity and are expressed by innate immune system cells and certain cancer cells [5,16]. There are ten TLR family members

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made up of type I transmembrane proteins. Indeed, TLRs can identify conserved features in microorganisms and trigger signal transduction pathways [17,18]. Toll-like receptor 4 (TLR4) is the first identified human TLR [18,19,20]. Genetic polymorphisms in TLRs, especially the TLR4 gene, have been revealed to be associated with a range of infectious diseases and cancers, including hematological malignancy. As a result, genetic variations in TLR4 gene may impair intracellular signaling in mononuclear cells, triggering immunological responses [18,21,22]. It is found that the TLR4 gene is located on chromosome 9 (9q33.1), containing three exons and encoding 839 residues of amino acids. TLR4 (rs4986791) is a missense polymorphism in the coding region of TLR4 gene exon 3, in which a C-T substitution occurs at position 1196 of the TLR4 gene resulting in a threonine to isoleucine change at 399 (Thr399Ile) [18,23]. Due to the controversial role of the TLR4 gene in AML, the current study aimed to investigate the variability and distribution of the single nucleotide polymorphism (SNP) variants of the TLR4 gene (rs4986791-Thr399Ile) among adult AML patients, referring to the association between the investigated SNP and the risk of AML as well as the risk of acquiring bacterial infection.

2. Materials and Methods

2.1. Study population

A total of 140 Egyptian participants were involved in this current study. Participants were divided into two groups: 80 AML patients and 60 controls. AML diagnosis was performed at the National Cancer Institute. Before blood sampling, written informed consent was received from each participant. All procedures in the study followed the World Medical Association's Declaration of Helsinki guidelines announced in 1975 and revised in 2008 for studies involving human subjects, as well as the guidelines of National Cancer Institute ethics committee, approval number CP2302-503-043.

Inclusion criteria: The group of patients included adult subjects of both genders and different ages. They were diagnosed to have AML, whereas the group of control individuals included adult healthy subjects of both genders and various adult ages with no evidence of cancer history. **Exclusion criteria:** Pediatric subjects were excluded from the sample recruitment. Subjects who tested positive for hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) were excluded from the study, regardless of whether they were patients or controls According to the FAB classification, AML patients were classified
into subtypes that included M1, M2, M3, M4, and M5. Control participants and patients were evaluated by routine clinical hematological parameters of complete blood picture, including total leucocyte count (TLC) referring to WBCs, hemoglobin (Hb), hematocrit [packed cell volume (PCV)], red blood cell cells (RBCs), and indices of RBCs [mean-corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)], and finally platelet count (PLC). The infections with pathogenic bacteria were identified in 24 out of 80 adult AML patients. These pathogenic microorganisms included *Klebsiella pneumonia, coagulase-negative Staph-ylococcus aureus,* and *Acinetobacter baumannii*.

2.2. DNA extraction from whole blood

The whole blood was withdrawn on the EDTA vacutainer tubes from all participants, and plasma was separated. The extraction of genomic DNA was performed according to the manufacturer's inst-ructions using genomic DNA extraction kits (Qiagen, Milan, Italy). The purified genomic DNA samples were quantified by ultraviolet absorbance at 260 nm through the use of a Thermo Scientific NanoDrop™ Spectrophotometer. The DNA was stored at -20 °C [24, 25].

2.3. Genotyping of TLR4 gene (rs4986791) polymorphism

Using a real-time PCR methodology based on the pre-validated TaqMan MGB™ probe for allelic discrimination assay (Applied Biosystems), the SNP in TLR4 (rs4986791) was identified. Briefly, 1.25 µL of a mix of 40X combined primer and probe (ABI/Life Technologies, USA) was carefully added to 12.5 µL of 2X TaqMan® Universal PCR Master Mix (ABI/Life Technologies, USA) to a final volume of 25 µL DNAse/RNAse-free water (Invitrogen/Life Technologies, USA) and template. The cycle conditions were as follows: 95 °C per 10 min, 95 °C per 15 sec, and 60 °C per 1 min. There were forty repetitions of the last two steps. The PCR run was conducted on a Rotor Gene real-time PCR system (Qiagen, Santa Clarita, CA). Allelic discrimination data was revealed by plots constructed in statistical package for social sciences (SPSS) version 16.0 (SPSS; Chicago,IL, USA) [24].

2.4. Statistical analysis

Data were collected initially using a specialized data collection form, then introduced into a Microsoft Excel worksheet and finally transferred to SPSS software version 25 (SPSS; Chicago, IL, USA) for analysis. The t test was used to analyze quantitative data. The chi-square test was used to analyze qualitative data. Finally, the logistic regression test was used to analyze the data to determine the association of independent variable(s) with one dich-otomous dependent variable and to dete-rmine an outcome. A *P*-value of ≤ 0.05 indicated significant results.

3. Results

3.1. Demographic information and baseline characteristics in the entire cohort of study

The demographic and hematological clinical features of the entire cohort of study including group 1 (control, $n = 60$) and group 2 (adult AML patients, $n = 80$), were listed in Table 1. A significant increase in age and TLC was reported among adult AML patients compared to controls ($P < 0.001$). Likewise, a significant change in gender was recoded (P 0.003). However, a significant decrease in Hb, PCV, RBCs, and PLC was recorded among adult AML patients compared to controls (P 0.006 for PLC and $P < 0.001$ for other parameters).

3.2. Distribution of TLR4 gene (rs4986791) polymorphism in healthy controls and adult AML patients

The allelic discrimination data curves regarding TLR4 (rs4986791) genetic polymorphism in the cohort of study are shown in Fig. 1 and Fig. 2. The distribution of the identified genotypes and alleles of TLR4 (rs4986791) among healthy controls and adult AML patients was listed and compared in Table 2. The TLR4 (rs4986791) genotype CC (wild-type) was the predominant genotype among adult AML patients and controls (95% and 93.33%, respectively), wher-eas the TLR4 (rs4986791) genotype CT was the less predominant genotype in this regard. Likewise, the C allele of the TLR4 gene (rs4986791) was the predominant allele among adult AML patients and controls (97.5% and 96.67%, respectively), whereas the T allele of this gene was the less predominant allele in this respect. No significant difference was obtained for the distribution of the TLR4 (rs4986791) genotypes or alleles in adult AML patients and controls. Furthermore, logistic regression analysis revealed a non-significant association between TLR4 (rs4986791) genetic polymorphism and the risk of AML among adult AML patients.

Table 1. Demographic data and baseline haematological clinical features among subjects of study

Parameter	Subjects of study						*P*-value
	Group 1 (Controls, *n* = 60)			Group 2 (AML patients, *n* = 80)			
Gender (M/F)	46/14			42/38			0.003*
Age (years)	28.866	±	7.280	38.825	±	12.271	< 0.001*
TLC (10^3 /cmm)	6.768	±	1.619	87.047	±	87.137	< 0.001*
Hb (g/dL)	14.504	±	1.450	7.842	±	1.396	< 0.001*
PCV (%)	43.225	±	5.380	23.464	±	3.909	< 0.001*
RBCs (10^6/cmm)	5.118	±	0.458	2.835	±	0.721	< 0.001*
MCV (fl)	84.179	±	3.984	85.208	±	11.579	0.278
MCH (pg)	33.586	±	0.820	28.358	±	3.895	0.439
MCHC (g/dL)	33.342	±	2.000	33.367	±	2.611	0.285
PLC (10^3 /cmm)	262.786	±	64.703	151.608	±	232.099	0.006*

Where: M: male, F: female, TLC: total leucocyte count (WBCs), Hb: hemoglobin, PCV: packed cell volume (hematocrite), RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLC: platelets count, and * = significant value. Normal ranges were as follows: TLC: from 4 to 11, Hb: from 14.00 to 17.5 for males and from 12.5 to 14 for females, PCV: 41 to 52, RBCs: from 4.5 to 6, MCV: 80 to 100, MCH: 27 to 33, MCHC: 31 to 37, and PLC: from 150,000 to 450,000. The t-test was applied to analyze the data for all parameters, except for the data of the gender parameter, which was analyzed using the chi-square test. Except for gender, all data were expressed as means and standard deviation values (M ± SD).

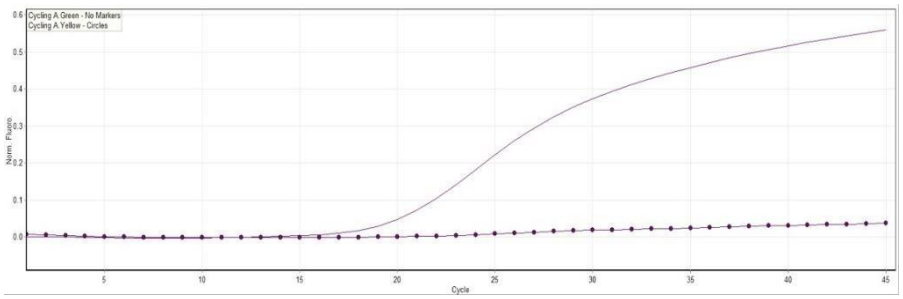

Fig.1. Allelic discrimination curve for the TLR4 gene (rs4986791) generated by the Rotor Gene real-time PCR system illustrating homozygous alleles (CC) bearing the FAM™ label. The x-axis represents the amplification cycle number, whereas the y-axis represents the fluorescence value.

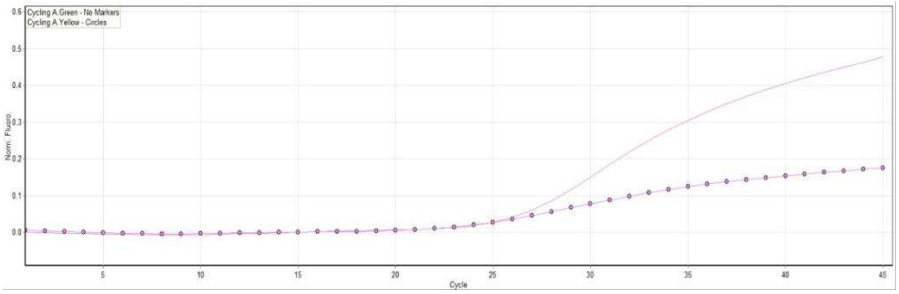

Fig.2. Allelic discrimination curve for TLR4 gene (rs4986791) generated by the Rotor Gene real-time PCR system illustrating heterozygous alleles (CT), where one is identified with the FAM™ and the other with VIC™ label. The x-axis represents the amplification cycle number, whereas the y-axis represents the fluorescence value.

Table 2. Distribution of TLR4 (rs4986791) genotypes and alleles in healthy controls and adult AML patients

TLR4 gene (rs4986791)	Subjects of study				Chi-square		Logistic regression		
	Group 1 (controls) *n*= 60		Group 2 (patients) *n* = 80						
	n	%	*n*	%	X^2	*P*-value	Odd's ratio	95% C.I.	*P*- value
CC genotype	56	93.33	76	95	0.177	0.674	0.737	0.1766 to 3.0737	0.675
CT genotype	4	6.67	4	5					
Total genotypes	60	100	80	100					
C allele	116	96.67	156	97.5	0.172	0.678	0.745	0.1822 to 3.0354	0.680
T allele	4	3.33	4	2.5					
Total alleles	120	100	160	100					

3.3. Distribution of TLR4 gene (rs4986791) polymorphism in different subtypes of AML among adult AML patients

The distribution of the identified genotypes and alleles of TLR4 (rs4986791) in AML patients in relation to different AML subtypes is presented in Table 3. AML patients were divided into 3 groups according to AML subtypes: group1 represented AML patients with M1 and M2 subtypes; group2 represented AML patients with M3 subtype; and group3 represented AML patients with M4 and M5 subtypes. Chi-square analysis revealed a non-significant change in the distribution of either genotypes (CC and CT) or alleles (C and T) among different groups of adult AML patients. The results referred to the predominance of the TLR4 (rs4986791) CC genotype and C allele in all AML groups. However, the TLR4 (rs4986791) CT genotype was absent in adult AML patients with M3 subtype.

Table 3. Distribution of TLR4 (rs4986791) genotypes in adult AML patients according to different AML subtypes

TLR4 gene (rs4986791)	AML patients, *n* (80)			Chi-square	
	Group1 [M1 and M2, *n* (50)]	Group2 [M3, *n* (2)]	Group3 [M4 and M5, *n* (28)]		
	n (%)	*n* (%)	*n* (%)	X^2	*P*-value
CC genotype	48 (96%)	2 (100%)	26 (92.86%)	0.481	0.819
CT genotype	2 (4%)	0 (0%)	2 (7.14%)		
Total genotypes	50 (100%)	2 (100%)	28 (100%)		
C allele	98 (98%)	4 (100%)	54 (96.43%)	0.412	0.814
T allele	2 (2%)	0 (0%)	2 (3.57%)		
Total alleles	100 (100%)	4 (100%)	56 100%)		

3.4. Relationship between TLR4 gene (rs4986791) polymorphism and risk of bacterial infection among adult AML patients

The distribution of the identified genotypes and alleles of TLR4 (rs4986791) in adult AML patients with respect to the risk of bacterial infection was listed in Table 4. Chi-square analysis referred to a non-significant change in the distribution of either genotype or allele among bacterial infected AML patients and non-infected patients. Moreover, logistic regression analysis indicated a non-significant association of TLR4 (rs4986791) genetic poly-morphism with the incidence and risk of bacterial infection among adult AML patients. The results referred to the predominance of the TLR4 (rs4986791) CC genotype and C allele among AML patients with bacterial infection, whereas the TLR4 (rs4986791) CT genotype and T allele were absent.

Table 4. Distribution of TLR4 (rs4986791) polymorphism in adult AML patients in relation to the risk of bacterial infection

TLR4 gene (rs4986791)	AML patients regarding bacterial infection				Chi-square		Logistic regression		
	Group 1 (infected) *n*= 24		Group 2 (non-infected) *n* = 56						
	n	%	*n*	%	X^2	*P*-value	Odd's ratio	95% C.I.	*P*-value
CC genotype	24	100	52	92.86	1.805	0.179	0.238	0.0123 to 4.5988	0.342
CT genotype	0	0	4	7.14					
Total genotypes	24	100	56	100					
C allele	48	100	108	96.43	1.825	0.177	0.249	0.0131 to 4.7082	0.354
T allele	0	0	4	3.57					
Total alleles	48	100	112	100					

4. Discussion

Different reports revealed that TLRs have a dual role effect in malignant diseases, the anti-tumoral effects owing to the immune response effectiveness and the pro-tumoral effects as a result of the pro-inflammatory signals, which produce cytokines and chemokines, in addition to anti-apoptotic substances, and growth factors that enhance proliferation of tumor cells and promote cancer metastasis [5,26,27]. Moreover, several studies have demonstrated that tumor cells express TLRs, which are implicated in carcinogenesis, tumor expansion, and metastasis. [5,28,29,30,31]. A considerable number of SNPs have been observed in the genetic makeup of these receptors. Indeed, TLR genes are polymorphic, therefore genetic changes in these genes can influence the etiology of several disorders. Previous studies have found some associations between TLR4 polymorphisms and susceptibility to infections, inflammation, and the risk of various malignancies [32,33,34,35]. TLR4 can interact synergistically with cells of innate immunity, therefore, it contributes to the inflammatory process, which enhances carcinogenesis in some conditions from a genetic outlook [36,37]. As TLR4 is expressed in lymphocytes and contributes to the regulation of B-cell differentiation and activation, thereby, genetic polymorphisms of the TLR4 gene may contribute to the etiology and pathology of leukemia [35,37,38]. There are few studies that highlight the relation between the SNPs of TLR4 and the risk of hematological malignancies [5]. According to a meta-analysis that included 55 articles, TLR4 gene polymorphisms, including TLR4 (rs4986791) and TLR4 (rs11536889), might represent a genetic risk for the development of cancer, but TLR4 (rs4986790) revealed no association with cancer [5,31]. The SNP of TLR4 (rs4986790) was found to be associated with the risk of mucosa-associated lymphoid tissue (MAL T) lymphoma and Hodgkin's lymphoma [5,39]. Consequently, and as a result of its controversial role, TLR4 rs4986791 genetic polymorphism was selected in our study to reveal the relation between this SNP and the risk of AML in adult patients.

It was established that the majority of patients with AML have increased serious levels of immature WBCs without sufficient number of RBCs or platelets in their blood [2,10]. Indeed, the findings of our study are entirely consistent with this confirmed fact. As our findings indicated, AML patients experienced a significant increase in TLC (WBCs), as well as a significant decrease in Hb, RBCs, and PLC compared with non-AML controls. In our study, a non-significant difference was obtained for the distribution of variant genotypes and alleles of the TLR4 (rs4986791) SNP among adult AML patients and healthy controls with the predominance of the homozygous CC genotype. Furthermore, no association was observed between the TLR4 (rs49 86791) SNP and the AML risk among these adult patients. Our findings agreed with those reported by Aref et al. [40], who recoded a non-significant difference between 120 AML patients and 100 controls regarding genetic variations in the TLR4 gene (rs 4986791) in a cohort of Egyptian patients. Conversely, our findings disagreed with those of Banescu et al. [5], who reported that variant genotypes of TLR4 (rs4986791) were associated with AML risk in their study of a large cohort of Eastern Europeans consisting of 511 AML cases and 503 healthy controls from Romania. Ethnic origin might be considered an explainable factor for the difference in the distributions of genotypes regarding TLR4 (rs4986791) in our study compared with that of Banescu et al. [5], who referred to AML in European subjects [5]. The significant role of TLR4 (rs4986791) in other types of cancer rather than AML was revealed by Moaaz et al. [41], who revealed a significant association between TLR4 gene (rs4986791) alleles and the risk of development of colorectal cancer in 268 Egyptian subjects (141 healthy controls and 127 patients) [5,41]. Additionally, our findings demonstrated no association between TLR4 (rs4986791) gene alleles and adult AML patients. This finding agreed with Banescu et al. [5], who found no association between the allele haplotypes of the SNP of TLR4 (rs498 6791) and adult AML patients [5]. During our study, the frequency of the TLR4 (rs4986791) SNP was further investigated among adult AML patients regarding different AML subtypes. Our results referred to a non-significant change in the distribution of the TLR4 (rs4986791) SNP genotypes (CC and CT) or alleles (C and T) among different groups of adult AML patient subtypes. Further, the wild-type CC genotype of TLR4 (rs4986791) was the predominant genotype among all groups, whereas the CT genotype was the less

predominant genotype, and it was completely absent in adult AML patients with the M3 subtype involved in group 2. Unfortunately, limited studies have investigated the distribution and association of the TLR4 (rs4986791) polymorphism with the AML subtypes. However, other studies investigated the relationship between other genetic polymorphisms and AML subtypes. Elderiny et al. [42], studied the relation between CYP2B6 gene polymorphism (rs3745274) and AML subtypes among AML Egyptian patients [42]. Furthermore, Xu et al. [43] studied the relationship between SNPs of the STAT3 gene (rs17886724 T/C and rs9909659 G/A) and AML subtypes among AML Chinese patients [43].

Generally, TLRs are capable of recognizing the conserved structures in microorganisms; these structures involved the pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), in addition to trans-membrane domains, and finally intracellular toll-interleukin 1 (IL-1) receptor (TIR) domains. As a result, TLRs can activate essential pathways of signal transduction. Several studies have demonstrated that the dysregulation of TLR signaling can lead to a variety of illnesses, including infectious diseases and malignancies [18,44]. In fact, TLR4, a type I transmembrane glycoprotein, has an extracellular domain containing 22 leucine-rich repeats (LRRs) structure that mediates the recognition of pathogen lipopolysaccharides (LPS). Ligand binding to this structure initiates TLR4 activation, triggering a cascade of signal transduction and a number of inflammatory pathways, mainly by means of the adaptor molecule myeloid-differentiation protein 88 (MyD88). Therefore, it activates the nuclear factor kappa beta (NF kB) pathway and enhances the transcription of pro-inflammatory cytokines like interleukin (IL) proteins, including IL-1, IL-6, and IL-8 [18,45,46]. Indeed, it was revealed that TLR4 polymorphisms might be incident in 10% Caucasian populations. These polymorphisms might be reported to have a positive correlation with acquiring several infections, involving Gram-negative bacterial sepsis [40,47,48]. Finally, TLR4 constitutes pathogen re-cognition receptors, which recognize lipopoly-saccharides of bacteria, especially for Gram-negative, mycobacterial, and fungal pathogens. Polymorphisms of the TLR4 gene (rs4986791) might alter the function of the receptor. Some studies have proposed that TLR4 gene (rs4986791) and (rs4986790) polymorphisms might lead to a reduction in cytokine response, increasing the susceptibility to Gram-negative bacterial infection [49]. However, other studies failed to reveal a significant association between TLR4 gene (rs4986791) and (rs4986790) polymorphisms and infectious events [49].

In our study, the relationship between the TLR4 gene (rs4986791) genetic polymorphism and the risk of bacterial infections among AML patients was investigated. A non-significant change in the distribution of the TLR4 (rs4986791) genotypes and alleles was observed among adult AML patients regarding the incidence of bacterial infections. Moreover, the genetic polymorphism of TLR4 (rs4986791) was not associated with the incidence or the risk of bacterial infection among adult AML patients. In general, bacterial infections mainly represent a main etiology for pneumonia and sepsis followed by fungal infections. In fact, there is a deficiency in the studies, which correlate TLR4 (rs4986791) with bacterial infections among adult AML patients. Our findings disagreed with those recorded by Schnetzke et al. [50], who found that the SNP of TLR4 (rs4986791-Thr399Ile) was an independent risk factor with an observed impact for pneumonia and sepsis development as complications of infection in a study that included 155 AML patients subjected to induction chemotherapy [50]. The above mentioned study findings might differ from our findings due to the induction of chemo-therapy among the studied population, whereas our study involved naïve treatment patients. Furthermore, our results disagreed with those reported by Aref et al. [40], who found that the CT genotype, and C allele SNP variants of TLR4 (rs4986791) were sig-nificantly associated with sepsis and pneumonia compared with other genotypes and alleles among a cohort of 120 AML patients [40]. Moreover, the relationship between the genetic polymorphism of TLR4 (rs4986791) and severe infections acquired by causative agents rather than bacterial pathogens was demonstrated. A previous study conducted by Chen et al. [51] revealed no association between TLR4 (rs4986791) polymorphism and the risk of devel-oping lung invasive fungal disease among 172 adult Chinese Han AML patients [51]. The limitation of the current study was the small sample size of adult AML patients, and consequently, the percentage of cases in each AML subtype is not balanced. Moreover, the absence of the mutant TLR4 (rs4986791) TT genotype in the studied cohort may be attributed to the lack of the mutant form in the Egyptian population [24].

5. Conclusion

Our findings showed that the wild-type homo-zygous (CC) variant of the TLR4 gene (rs4986791-Thr399Ile) is the predominant genotype among adult AML patients with no difference from healthy controls. Genetic polymorphism of the TLR4 gene (rs4986791) does not appear to be associated with AML risk, AML different subtypes, and the risk of bacterial infection in the studied AML cohort. The future studies are recommended to investigate the correlations of TLR4 gene (rs4986791) polymorphism side by side to TLR4 gene expression analysis on a larger sample size of a population of adult AML patients, which may provide additional beneficial information regarding AML pathology among adults.

6. Abbreviations

AML: Acute Myeloid Leukemia
FAB:(French-American-British) classification system
SNP: Single Nucleotide Polymorphism
TLR4: Toll-like Receptor 4
WBCs: White Blood Cells

7. Author contributions

Ahmed Khedr: Conceptualization, methodology, data collection and analysis, data interpretations, writing the manuscript, and revision of important contents.

Mai Abd El-Meguid: Methodology and data collection.
Rania S. Abdel Aziz and Enas M. Radwan: Patient recruitment, and participation in methodology, data collection and analysis.
Reham Dawood: Conceptualization, data interpretations, and revision of important contents.

8. Conflict of interest

The authors declared that they have no conflict of interest.

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