Original Article

Investigation the Association Between Parvovirus B19 and Toll-like Receptor 3 Gene Polymorphism in Iraqi Patients with Chronic Myeloid Leukemia

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ABSTRACT

Objective: To investigate Parvovirus B19 infection and tool-like receptor-3 gene polymorphism in Iraqi patients with chronic myeloid leukemia.

Study Design: Case control study

Place and Duration of Study: This study was conducted at the College of Science, University of Babylon, Iraq from 1st August 2025 to 31st December 2025.

Methods: One hundred freshly whole bloods were obtained from patients with chronic myeloid leukemia while control group included 100 freshly whole bloods. Viral and total deoxyribonucleic acid genomic extraction were done to detection the B19 by polymerase chain reaction technique and toll-like receptor-3 gene polymorphism by sequencing, respectively

Results: B19 deoxyribonucleic acid was not detected in any patient sample. The results showed that deoxyribonucleic acid polymorphism distribution were deoxyribonucleic acid polymorphism distributions according to major homozygous genotype, heterozygous genotype and minor homozygous genotype were 76.5%, 17.6%, and 5.9%, respectively, in patients with chronic myeloid leukemia and 42.9%; 57.1%and 0.00%, respectively in control group

Conclusion: Toll-like receptor-3 may play a role in the tumor biology of the examined subset of chronic myeloid leukemia and may contributed to their development

Key Words: Chronic myeloid leukemia, Polymerase chain reaction, Toll-like receptor-3, Sequencing

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INTRODUCTION

Chronic myeloid leukemia (CML) is defined as a clonal myeloproliferative neoplasm originating from the hematopoietic stem cell.[1] The disease expresses the Philadelphia chromosome as a result of a reciprocal translocation between chromosomes 9 and 22 that creates the BCR-ABL1 fusion gene, which codes for a tyrosine kinase is always active. Chronic myeloid leukemia is grouped into three phases based on the percentage of blasts in the bone marrow or peripheral blood: chronic, accelerated, and blast crisis.

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Among other environmental factors that have been linked to the development of leukemia are smoking, exposure to electromagnetic fields, use of hair dye, exposure to organic solvents, and viral infections. Chronic myeloid leukemia is generally not considered a hereditary disease, and no clear genetic predisposition has been identified. Parvovirus B19 (PVB19) belongs to the family Parvoviridae, genus Erythrovirus.[2,3] It is a small, single-stranded DNA virus. Modes of transmission include respiratory secretions, blood transfusion, hand-to-mouth contact, and transplacental route. Infection is largely asymptomatic but can present with mild symptoms such as erythema infectiosum and arthralgia. The reported prevalence ranges from 4-72% in patients with hematological malignancies, reflecting the tropism of the virus for erythroid progenitor cells.[3,4] Toll-like receptor 3 (TLR3) is a receptor of innate immunity that detects viral double-stranded RNA. It is expressed in endocytic compartments and in several immune and epithelial cells.[5,6] Toll-like receptor-3 signaling has been associated with tumor progression, carcinogenesis, and regulation of inflammatory pathways through NF-κB and related mediators; it also has dual regulatory effects in the p53 pathway.[7,8]

Therefore, the present study purpose is to investigate B19 infection and TLR3 gene polymorphism in Iraqi patients with chronic myeloid leukemia.

METHODS

This case control study was conducted at College of Science, University of Babylon, Iraq from 1st August 2025 to 31st December 2025 vide letter No. M250213/QM/Approval/JSDJNEHU dated 19th July 2025. The study was conducted on two hundred specimens, 100 specimens were collected from patients with CML from Heamatological centers in the Middle Euphrates region of Iraq, and 100 specimens from apparently healthy controls (AHC) from the Babylon population. The age of patients and AHC groups ranged from 20 to 65 years.

The viral genome was extracted from blood samples using a commercial DNA/RNA extraction kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. Extracted DNA was stored at −20°C until PCR analysis. The primer sequences used for B19 detection were 5′-AATGCAGATGCCCTCCACG-3′ and 5′-ATGATTCTCCTGAACTGGTCCA-3′, generating a 493 bp product.

Genomic DNA was extracted from whole blood samples using the G-Spin™ Total DNA Extraction Mini Kit (Intron, Korea) following the manufacturer's protocol. Purified DNA was used directly for PCR amplification and stored at −20°C until use. TLR3 primers were: Forward GCCACACACTTCCCTGATGA and Reverse TGTCTCCCCTCCAGCCAATA, producing a 498 bp amplicon at an annealing temperature of 59°C.

Polymerase chain reaction products were subjected to Sanger sequencing. Obtained sequences were analyzed using chromatograms to identify nucleotide variations and determine TLR3 polymorphisms.

Data were analyzed using SPSS-25. Chi-square test was applied and $p<0.05$ was considered significant.

RESULTS

The age of patients' groups were matching with control group (p=0.57). The sex ratio among new diagnosis group and treated group was 1.5 and 1.08 respectively which was match with control group ratio 1.5(p=0.59) [Table 1). Most biochemical parameters showed no significant differences among groups, except creatinine levels between newly diagnosed and relapse patients (Table 2).

The TLR3 genetic sequences located on chromosome 16 were targeted. The 463 bp amplicon analyzed in this research represents part of the exon region of the TLR3molecule, which plays a key role in B cell development and activation. When analyzed using the NCBI BLASTn engine, the obtained sequence showed approximately 99% similarity to the human TLR3 reference sequence (GenBank acc. NC-000016.10) [Fig. 1].

Table No. 1: Age and sex distribution between patients with CML and AHC group (n=200)

Parameters	Patients (n=100)	Controls (n=100)	P value
Age (years)	49±12.43	46±12.9	0.57
Gender			
Male	52 (52%)	56 (56%)	0.59
Female	48 (48%)	44 (44%)	

Table No. 2: Estimation the levels of some biochemical parameters for new diagnosis CML, treated patients respond to treatment and relapse groups

Parameters	New diagnosis (n=20)	Treated (n=100)		P value
		Response to treatment (n=69)	Relapse (n=31)	
Urea (mg/dl)	31.5±7.8	33.3±18.2	30.3±12.1	*0.615 **0.69 ***0.399
Creatinine(mg/dl)	0.71±0.51	1.0±0.73	0.9±0.33	*0.059 **0.01 ***0.329
ALT (IU/L)	29.5±10.9	30.5±10.79	28.3±12.3	*0.73 **742 ***418
AST (IU/L)	33.6±12.6	32.2±12.7	33.2±15.6	*0.671 **931 ***733
LDH (IU/L)	478±201	485±278	438±20.4	*0.909 **0.49 ***0.39

*Comparison between new diagnosis CML and response to treatment patients. **Comparison between new diagnosis CML patients and relapse patients. ***Response to treatment patients and relapse patients

Table 3: Genotyping of TLR3 gene in CML patients and AHC groups

Genotype/ allele	Patients No. (% of called)	Controls No. (% of called)	Chi-square	p-value	Odds ratio (95% CI)	SNP type
G/G	13 (76.5%)	3 (42.9%)	0.0	1.0	1.0	Intron variant
G/A	3 (17.6%)	4 (57.1%)	3.39	0.066	0.17 (0.02-1.22)	Intron variant
A/A	1 (5.9%)	0 (0.0%)	0.23	0.633	0.78 (0.03-23.53)	Intron variant
Allele G	29	10	0.0	1.0	1.0	Intron variant
Allele A	5	4	1.25	0.263	0.43 (0.10-1.93)	Intron variant
Unconfirmed/ excluded	0	0	Not counted	Not counted	Not counted	Weak/conflicting/ no-call trace

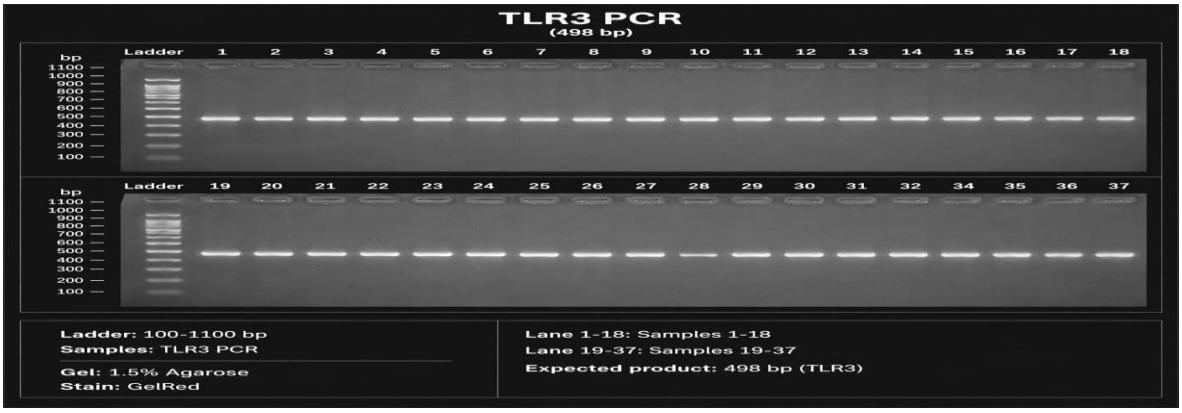

Figure No. 1: Agarose gel electrophoresis of PCR-amplified TLR3 fragments

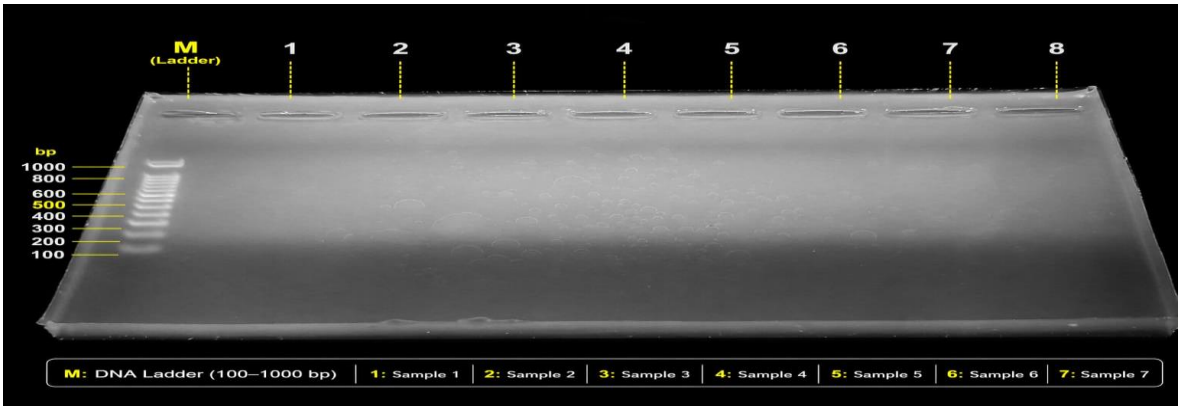

Figure No. 2: Agarose gel electrophoresis of PCR products for B19 DNA in CML patients. M: 100–1100 bp DNA ladder. Samples were separated on 2% agarose gel at 75 V and 20 mA for 120 min, showing no detectable B19 amplification

Table No. 4: The results of PCR for B19-DNA-infection in patients with CML

B19	No.	%
Positive	-	-
Negative	100	100.0

TLR3 genotype frequencies were GG 76.5%, GA 17.6%, and AA 5.9% in patients and 42.9%, 57.1%, and 0.0% in controls. Allele frequencies showed no significant difference between groups (Table 3).

Amplification detection of B19 by PCR technique in samples from patients with CML, B19 DNA was not detected in patients or controls (Table 4, Fig. 2). Spearman's Rho statistical testing of age, sex, B19; SNPs of TLR3in the Studied Population Groups, a significant correlation was observed between TLR3 polymorphism and age (r=0.722, p=0.03), whereas no correlation was found with B19 DNA (Table 5).

Table 5: Spearman's Rho statistical testing of age, sex, B19-DNA-PCR andTLR3 SNP to evaluate the studied markers in patients with CML

Spearman's rho		Age	TLR3 polymorphism	Sex
TLR3 Polymorphism	r	0.722		0.342
	p	0.03		0.6
B19-DNA	r	0.399	0.151	
	p	0.8	0.7	

DISCUSSION

Chronic myeloid leukemia (CML) is a condition in which the bone marrow makes too many white blood cells. It is one type of cancer of the blood and bone marrow. In this study, the age and sex distributions of the patients and the control group were not significantly different (p=0.57 and p=0.59, respectively). These results are in line with other studies that have shown chronic myeloid leukemia to commonly afflict adults with median ages ranging between 35 and 64 years and a slight male predominance.[9,10]

Berger et al[11] reported gender-related differences in CML characteristics: higher platelet counts and smaller spleen size in females; a greater frequency of additional chromosomal abnormalities and poorer survival in males. These differences were not observed in the current study. Most parameters showed no significant differences among the patient groups. Only serum creatinine showed a difference between newly diagnosed and relapse patients. Liver and kidney function markers might be related to disease progression or adverse effects of tyrosine kinase inhibitors (TKIs), including imatinib, dasatinib, and nilotinib. No sex-related differences were observed in this study. A similar trend was not found in the present study. Serum creatinine showed significant difference between newly diagnosed and relapse patients. Liver and kidney function markers might be related to disease progression or adverse effects of tyrosine kinase inhibitors (TKIs), including imatinib, dasatinib, and nilotinib.

Toll-like receptor-3 signaling pathways regulate the survival and proliferation of myeloid leukemia cells. TLR3 shares little in common with other TLRs in terms of downstream signaling, but components of the IRAK1/4–NF-κB pathway are critical for leukemic cell survival. In previous research, it has been shown that myeloid malignancies' growth could be suppressed and apoptosis induced upon IRAK1/4 inhibition, hence supporting these pathways as potential therapeutic targets.[12-14] Results of the current study showed the prevalence of the GG genotype in CML patients. Although allele A was more frequent in patients, no significant association was found. Similar results provide evidence that the dysregulation of TLR3, through inflammatory and immune-related pathways, may contribute to leukemogenesis and disease progression.[15] The prevalence of parvovirus B19 varies depending on geographical region and study population, with reported rates ranging between 20% and 60% in published papers.[16,17] In stark contrast, B19 DNA was not found in any of the patient samples in our study. This difference may be due to variations in population characteristics, sample size, viral circulation, or timing of detection. A significant correlation was seen between TLR3 polymorphism and patient age. No significant association was found between TLR3 polymorphism and B19 infection. Previous studies have demonstrated increasing B19 seroprevalence with age, probably reflecting cumulative exposure over time.[18] Overall, the findings suggest that TLR3 polymorphism may contribute to the biology of CML. Parvovirus B19 does not seem to play a significant role in the studied population.

CONCLUSION

Toll-like receptor-3 may play a role in the tumor biology of the examined subset of chronic myeloid leukemia and may contributed to their development.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa Khalid Saleh, Shakir H. Mohammed Al-Alwany
Drafting or Revising Critically:	Zahraa Khalid Saleh, Alaa Tariq Shaker
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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