Original Article

Genetic Polymorphism of TLR-4 and CD-14 in Patients with Chronic Prostatitis

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Abstract

Background: Prostatitis is the medical term for inflammation of the tissue in the prostate gland. It is recognized to be associated with innate immunity since several cytokines are implicated in the occurrence and progression of Chronic abacterial prostatitis/chronic pelvic pain syndrome. **Objectives:** Study the relationship between immune status involving genetic polymorphism for host–microbes interacting receptors and the occurrence of chronic prostatitis (CP). **Materials and Methods:** This case–control study involved a population of CP patients and healthy individuals. Full information was taken from each subject. A total of 40 samples were collected from patients diagnosed with CP according to their clinical manifestations, with an age range of 17–62 years. Patients were diagnosed by a consultant urologist. A total of 50 venous blood samples were taken from apparently healthy persons to serve as the control group. The study was conducted between December 2022 and June 2023, within the Urology Department and Microbiology Department in the College of Medicine, University of Babylon, and Hilla Teaching Hospital. **Results:** The mean age of patients with CP was 35.57 ± 8.81 years, while that of control subjects was 33.24 ± 9.55 years. There was no significant difference in mean age between patients with CP and control subjects (P = 0.236). The distribution of *TLR4-rs11536889-G/C*, *TLR4-rs1927911-C/T*, *CD14-rs5744454-C/A*, and *CD14-rs2569190-T/C* polymorphisms was detected using the Tetra-amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR) technique. Regarding genotype mode, there was a non-significant difference in the frequency distribution of genotypes between patients with CP and healthy controls. **Conclusion:** The results revealed a significant association between types of infection and results of T-ARMS-PCR (P = 0.022). However, there were no significant differences in the frequency distribution of genotypes of toll-like receptor 4 (TLR-4) and cluster of differentiation 14 (CD-14) between patients with CP and healthy controls. Also, the results indicated a non-significant association between types of infection and gene polymorphism.

Keywords: CD-14, chronic prostatitis, polymorphism, TLR-4

Introduction

Prostatitis is the medical term for inflammation of the tissue in the prostate gland. It may happen as a necessary physiological reaction to an infection or it may happen in the absence of an infection. It is characterized by potentially excruciating pain and distress, though it usually improves with time.[1]

Toll-like receptor 4 (TLR-4), encoded by the TLR-4 gene in humans, is a transmembrane protein and a member of the pattern recognition receptors (PRRs) family. It is predominantly expressed in myeloid cells such as macrophages, erythrocytes, and granulocytes, rather than in lymphoid cells such as T-cells, B-cells, and NK cells.[2]

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TLRs, examples of PRRs, are named for their ability to identify pathogen-associated molecular patterns (PAMPs), which are components found in various microorganisms such as bacteria, viruses, fungi, and parasites. The interaction between PRRs and PAMPs is crucial for initiating rapid inflammatory responses required for innate immunity and for enhancing adaptive immunity by

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stimulating cytokine production. This process is essential for establishing effective immunity.[3]

Invading pathogens are recognized by toll-like receptors, initiating both innate immune reactions and boost adaptive immunological reactions. Upon initial attachment to the lipopolysaccharide (LPS)-binding protein (LBP) and CD14, pathogens interact with the TLR-4/MD-2 complex, activating the TLR-4 cell signaling pathway. Activation of the TLR-4/MD-2 complex induces conformational changes, facilitating downstream signal transduction processes.[4]

The human protein CD14 is primarily produced by macrophages and serves as a key component of the innate immune system. By binding to LPS, a PAMP, CD14 aids in the detection of pathogens in the body.[5] There are two types of CD14: the mCD14 form, which is found on the surface of monocytes, dendritic cells, neutrophils, and macrophages, is anchored to the membrane by a glycosylphosphatidylinositol tail. Other is soluble form (sCD14), either a form with a high molecular mass of 53–56 kDa or a form with a low molecular mass of 48–50 kDa.[6]

The accessory proteins CD14 and MD-2 are involved in the ligand recognition, dimerization, and endocytosis of TLR-4 when the LBP binds to LPS and transfers it to those proteins. After dimerization, TLR-4 can signal through either the MyD88-dependent or -independent pathway which leads to produce pro-inflammatory cytokines.[7]

cluster of differentiation 14 (CD-14), in collaboration with MD-2 and TLR-4, serves as a co-receptor for the detection of bacterial LPS. However, CD14 can only bind to LPS in the presence of LBP. While LPS is considered its principal ligand, CD14 is also capable of recognizing other PAMPs, such as lipoteichoic acid.[6] Inflammatory disorders and risk factors have been associated with elevated levels of sCD14, which is produced in response to both acute and chronic inflammatory situations.[8] The aim of this study is to investigate gene polymorphism in patients with chronic prostatitis (CP) and its association with microbial infection. Additionally, we aim to explore the role of TLR-4 and CD-14 polymorphism in the induction of CP.

Materials and Methods

Study design and patients

The present work is a case–control study, involving a population of CP patients and healthy individuals. Full information was apparently taken from each subject. A total of 40 samples were collected from patients suspected of having CP based on their clinical manifestations, with an age range of 17–62 years. Additionally, 50 apparently healthy individuals served as the control group. Patients were diagnosed by consultant urologist, in Babylon province, Iraq. The distribution of TLR-4 and CD-14 single nucleotide polymorphisms (SNPs) was detected using Tetra-ARMS-PCR technique.

Statistical analysis

Data were collected, summarized, analyzed, and presented using SPSS version 26 (SPSS Inc., Chicago, Illinois) and Microsoft Office Excel 2010. Numeric data were presented as mean and standard deviation after conducting the Kolmogorov–Smirnov normality test and determining the distributed values of variables as normal and non-normal. The Chi-square test was used to study the association between any two categorical variables.

Ethical approval

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Prior to sample collection, verbal and written informed consent was obtained from all patients. The study protocol, the subject information, and consent form were reviewed and approved by a local ethics committee of Babylon Medical College according to document number 78, in November 2022.

Results

The present study enrolled 40 patients with CP and 50 healthy control subjects. The mean age of patients was 35.57 ± 8.81 years, while that of control subjects was 33.24 ± 9.55 years. There was no significant difference in mean age between patients with CP and control subjects (P = 0.236). The distribution of *TLR4-rs11536889-G/C* polymorphism was detected using the ARMS-PCR technique. Three genotypes (GG, GC, and CC) are observed at this locus. There was no significant difference in the frequency distribution of genotypes between patients with CP and healthy control (P = 0.576), as shown in Table 1. The present results revealed a significant association between types of infection and

Table 1: *TLR4-rs11536889-G/C* polymorphism genotype frequency in patients with CP and healthy controls

TLR-4 (rs11536889)	Patients (n = 40)	Control (n = 50)	P	OR	95% CI
Genotype frequency					
CC	6 (15.0%)	4 (8.0%)	0.576	2.02	0.51-7.89
G/C	8 (20.0%)	11 (22.0%)	¥ NS	0.97	0.34 -2.77
GG	26 (65.0%)	35 (70.0%)		Reference	
Allele frequency					
C	20 (25.0%)	19 (19.0%)	0.331	1.42	0.69-2.89
G	60 (75.0%)	81 (81.0%)	¥ NS	Reference	

the outcomes of T-ARMS-PCR ($P = 0.022$), as indicated in Table 2.

The distribution of *TLR4-rs1927911-C/T* polymorphism revealed three genotypes (CC, CT, and TT) at this locus. Analysis of genotypic modes showed a non-significant difference in the frequency distribution between patients with CP and healthy controls ($P = 0.304$), as presented in Table 3. The present results indicated a non-significant association between types of infection and the outcomes of T-ARMS-PCR ($P = 0.201$), as observed in Table 4. The distribution of *CD14-rs5744454-C/A* polymorphism displayed three genotypes (CC, CA, and AA) at this locus. Analysis of genotypes modes revealed a non-significant difference in the frequency distribution between patients with CP and healthy controls ($P = 0.112$), as shown in Table 5.

The present results indicated a non-significant association between types of infection and the outcomes of T-ARMS-PCR ($P = 0.075$), as observed in Table 6. The distribution of *CD14-rs2569190-T/C* polymorphism revealed three genotypes (TT, TC, and CC) at this locus. Analysis of genotypes mode showed a non-significant difference in the frequency distribution between patients with CP and healthy controls ($P = 0.112$), as shown in Table 7. The present results revealed a non-significant association between types of infection and the outcomes of T-ARMS-PCR ($P = 0.569$), as shown in Table 8.

Table 2: Comparison of frequency distribution of type of infection in patients with CP according to the results of *TLR4-rs11536889-G/C* polymorphism

Characteristic	Bacterial infection ($n = 2$)	HSV-2 infection ($n = 31$)	Mixed infection ($n = 6$)	*P*
Genotype				
GG genotype, *n* (%)	1 (50.0%)	23 (74.2%)	1 (16.7%)	0.022 ¥ S
GC genotype, *n* (%)	0	6 (19.4%)	2 (33.3%)	
CC genotype, *n* (%)	1 (50.0%)	2 (6.5%)	3 (50.0%)	

Table 3: *TLR4-rs1927911-C/T* polymorphism genotype frequency in patients with CP and healthy controls

TLR4-(rs1927911)	Patients ($n = 40$)	Control ($n = 50$)	*P*	OR	95% CI
Genotype frequency					
TT	4 (10.0%)	3 (6.0%)	0.304 ¥ NS	2.2	0.44–10.86
C/T	16 (40.0%)	14 (28.0%)		1.89	0.76–4.67
CC	20 (50.0%)	33 (66.0%)		Reference	
Allele frequency					
T	24 (30.0%)	20 (20.0%)	0.120 ¥ NS	1.71	0.86–3.40
C	56 (70.0%)	80 (80.0%)		Reference	

Table 4: Comparison of frequency distribution of type of infection in patients with CP according to the results of *TLR4-rs1927911-C/T* polymorphism

Characteristic	Bacterial infection ($n = 2$)	HSV-2 infection ($n = 31$)	Mixed infection ($n = 6$)	*P*
Genotype				
CC genotype, *n* (%)	2 (100.0%)	16 (51.6%)	2 (33.3%)	0.201 ¥ NS
CT genotype, *n* (%)	0	13 (41.9%)	2 (33.3%)	
TT genotype, *n* (%)	0	2 (6.5%)	2 (33.3%)	

Discussion

The mechanism by which the *TLR4-rs11536889-G/C* polymorphism is associated with CP is not fully understood. However, it is thought that the polymorphism may affect the immune system's response to infection. The TLR-4 protein is a receptor that recognizes bacterial LPS, a major component of the outer membrane of gram-negative bacteria. Upon activation, TLR-4 initiates a cascade of events leading to the production of inflammatory cytokines.[9,10] It is possible that the *TLR4-rs11536889-G/C* polymorphism alters the sensitivity of TLR-4 to LPS, potentially resulting in an

Table 5: *CD14-rs5744454-C/A* polymorphism genotype frequency in patients with CP and healthy controls

CD14-(rs5744454)	Patients ($n = 40$)	Control ($n = 50$)	*P*	OR	95% CI
Genotype frequency					
AA	4 (10.0%)	2 (4.0%)	0.112 ¥ NS	3.23	0.55–18.9
C/A	10 (25.0%)	6 (12.0%)		2.69	0.87–8.28
CC	26 (65.0%)	42 (84.0%)		Reference	
Allele frequency					
A	18 (22.5%)	10 (10.0%)	0.021 ¥ S	2.61	1.13–6.04
C	62 (77.5%)	90 (90.0%)		Reference	

Table 6: Comparison of frequency distribution of type of infection in patients with CP according to the results of *CD14-rs5744454-C/A* polymorphism

Characteristic	Bacterial infection ($n = 2$)	HSV-2 infection ($n = 31$)	Mixed infection ($n = 6$)	*P*
Genotype				
CC genotype, *n* (%)	0	22 (71.0%)	4 (66.7%)	0.075 ¥ NS
CA genotype, *n* (%)	2 (100.0%)	5 (16.1%)	2 (33.3%)	
AA genotype, *n* (%)	0	4 (12.9%)	0	

Table 7: *CD14-rs2569190-T/C* polymorphism genotype frequency in patients with CP and healthy controls

CD14-(rs2569190)	Patients ($n = 40$)	Control ($n = 50$)	*P*	OR	95% CI
Genotype frequency					
CC	6 (15.0%)	3 (6.0%)	0.363 ¥ NS	2.81	0.64–12.25
T/C	7 (17.5%)	9 (18.0%)		1.09	0.36–3.30
TT	27 (67.5%)	38 (76.0%)		Reference	
Allele frequency					
C	19 (23.7%)	15 (15.0%)	0.136 ¥ NS	1.77	0.83–3.74
T	61 (76.3%)	85 (85.0%)		Reference	

Table 8: Comparison of frequency distribution of type of infection in patients with CP according to the results of *CD14-rs2569190-T/C* polymorphism

Characteristic	Bacterial infection ($n = 2$)	HSV-2 infection ($n = 31$)	Mixed infection ($n = 6$)	*P*
Genotype				
TT genotype, *n* (%)	1 (50.0%)	20 (64.5%)	5 (83.3%)	0.569 ¥ NS
TC genotype, *n* (%)	1 (50.0%)	6 (19.4%)	0	
CC genotype, *n* (%)	0	5 (16.1%)	1 (16.7%)	

exaggerated production of inflammatory cytokines. This dysregulated immune response could contribute to the chronic inflammation characteristic of CP.

Our findings are supported by a study,[10] which identified TLR-4 SNPs, such as rs4986791 and rs115336889, as risk and prognostic markers for benign prostatic hyperplasia (BPH). This study suggests that these markers may interact with environmental factors, such as alcohol intake, to increase an individual's susceptibility to BPH.

One possible explanation is that the TLR-4 polymorphism is associated with differences in the microbiome of the prostate gland, which refers to the community of microorganisms inhabiting it. It is possible that the TLR-4 polymorphism could alter the composition of the microbiome, rendering certain individuals more susceptible to certain types of infections. Additionally, the *TLR4-rs1927911-C/T* polymorphism may alter the sensitivity of TLR-4 to LPS, resulting in different levels of inflammation in response to infection.[1]

TLR gene polymorphisms have the potential to influence the ratio of pro- inflammatory to anti-inflammatory cytokines, thereby impacting an individual's susceptibility to infections, chronic inflammation, and cancer.[11]

While a study[12] found no association between SNP rs1927911 and prostate cancer, another investigation[13] identified rs1927911 as a significant predictor of prostate cancer risk.

The CD14-rs5744454 polymorphism is a complex genetic marker, and its involvement in CP is not fully understood. However, the rs5744454 polymorphism in the CD14 gene is a variant that may be associated with differential CD14 expression and immune responses induced by LPS.

The study reported that the CD14 polymorphism was associated with higher levels of pro-inflammatory cytokines in response to LPS stimulation, which may contribute to chronic inflammation.[14]

A study[15] has shown that the prostate cancer has been linked to the CD14 polymorphism, supporting the notion that inflammatory genetic variations may increase the risk of prostate cancer in African American men.

CD14 serves as a membrane receptor for LPS, playing a crucial role in innate immunity and the inflammatory response. One pathogenesis-related CD14 gene variant, 159C/T (rs2569190), has been identified, which may alter the protein's structure and function, thus influencing CD14-LPS interactions and sCD14 levels. These alterations can impact the immune response to infection and the clearance of the prostate from microbial infection.[16]

The CD14 gene contains several SNPs, with the CD14 159C/T (rs2569190), also known as CD14 260C/T, garnering the most attention for its potential association with cancer susceptibility, including gastric cancer, prostate cancer, lymphoblastic leukemia, and others.[17] Reference[18] demonstrated that TLR-4 SNPs and *Trichomonas vaginalis* infection in Iraqi women are not significantly correlated. Several local studies found a strong correlation between TLR-3 polymorphism and the probability of Hepatitis C virus infection among iraqi population.[19-21]

Conclusion

There was a non-significant difference in the frequency distribution of genotypes of TLR-4 and CD-14 between patients with CP and healthy controls. Also, the present results indicate a non-significant association between types of infection and results of gene polymorphisms.

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Conflicts of interest
There are no conflicts of interest.

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