TLR-4 Polymorphisms Shows The Genetic Susceptibility to Toxoplasmosis and Polycystic Ovary Syndrome

Haider Iskandar Flayh [1*], Al-Marsomy [1], Huda Dhaher [1], Khazaali, Enas Adnan Abdulrasol [2]

Abstract

Background: Toxoplasmosis, caused by Toxoplasma gondii, presents asymptomatic in healthy individuals but can lead to severe outcomes in immunocompromised individuals. Toll-like Receptors (TLRs) are crucial in recognizing pathogens like T. gondii. Specifically, TLR-4 gene polymorphisms (rs4986790, rs4986791) may influence susceptibility to toxoplasmosis and polycystic ovary syndrome (PCOS). Methods: A case-control study of 150 women categorized into PCOS + toxoplasmosis, PCOS only, toxoplasmosis only, and control groups. TLR-4 SNPs were examined using ARMS PCR. Results: TLR-4 rs4986790 showed no significant difference between PCOS + toxoplasmosis and PCOS only but correlated significantly with toxoplasmosis alone. Rs4986791 showed no significant correlation. Notably, the AG genotype of rs4986790 was more frequent among toxoplasmosis patients than controls, suggesting susceptibility. Discussion: Limited global research on this topic indicates conflicting findings. The rs4986790 SNP may affect TLR4 function, potentially through altered signaling pathways or ligand binding. Mutant TLR4's conformational changes could disrupt ligand docking and signaling pathways, reducing the immune response. Conclusion: The presence of the G allele within TLR-4 rs4986790 polymorphism may decrease TLR-4's interaction with T. gondii, reducing the immune response and increasing susceptibility to infection among AG genotype carriers.

Keywords: Toxoplasmosis, Polycystic ovary syndrome, Toll-like receptor 4, Gene polymorphisms, Susceptibility

Significance | In this study TLR-4 polymorphism showed a role on susceptibility to Toxoplasma gondii infection and polycystic ovary syndrome development.

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Introduction

Toxoplasmosis infection is typically asymptomatic in individuals with a fully functioning immune system. However, in those with compromised immunity, it can lead to severe or even fatal manifestations (Elmore et al., 2010). Toxoplasmosis is caused by Toxoplasma gondii (T. gondii), an obligate intracellular parasite that is widely recognized as one of the most prevalent global parasites (Tenter et al., 2000).

Toll-like receptors (TLRs) are a class of transmembrane signaling receptors that play a pivotal role in orchestrating both innate and adaptive immune responses. They are integral to the regulation of inflammatory processes and the activation of immune cells aimed at eradicating infectious agents and cancerous cells (Iwasaki & Medzhitov, 2004). Up to this point, researchers have identified ten distinct types of TLRs in humans, each with the capacity to recognize various pathogens or molecular components (Theodoropoulos et al., 2010).

The TLR4 gene exhibits a high degree of polymorphism, with fifteen distinct polymorphic variations identified within its coding sequence. Among these single nucleotide polymorphisms (SNPs),

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two specific SNPs, rs4986790 and rs4986791, have been observed. Extensive research has been conducted to investigate the association between these SNPs and their potential role in conferring susceptibility to toxoplasmosis (Wujcicka et al., 2014). It is well-known that TLR/MyD88 signaling is a pivotal pathway in the non-specific antimicrobial response against T. gondii (Zare-Bidaki et al., 2014).

Additionally, it has been established that the glycosylphosphatidylinositol (GPI) present in T. gondii serves as a trigger for the TLR4 signaling pathways (Larsen et al., 2007). Infection with T. gondii stimulates the production of interferon gamma (IFNγ) through the activation of TLR4 and MyD88 signaling pathways. IFNγ is a multifunctional cytokine known to initiate inflammation, and this inflammatory response has downstream consequences on ovulation, implantation, and fertilization (Guo et al., 2015; Khan et al., 2020).

Zangeneh and colleagues have elucidated the pivotal role of immunity in the intricate processes of fertilization and implantation within the uterine environment (Zangeneh et al., 2017). Additionally, Escobar-Morreale and his research team have identified factors influencing pro-inflammatory responses and linked these factors to their correlation with the HPA (Hypothalamus-Pituitary-Adrenal) axis, which regulates adrenal steroidogenesis (Glintborg, 2016).

Polycystic ovary syndrome (PCOS) is characterized by an abnormal elevation in androgens, especially the male sex hormone testosterone, which is found in women in limited quantities. The term "polycystic ovary syndrome" denotes the presence of multiple small cysts, or fluid-filled sacs, forming within the ovaries (Zhao et al., 2015, Zeba et al. 2024, Zainab et al. 2024). Research in reproductive biology has revealed that pro-inflammatory cytokines, such as TNF-α, IL-6, and IFN-γ, influence ovarian function as well as the key processes of ovulation, fertilization, and implantation in individuals with PCOS. Conversely, anti-inflammatory cytokines, including IL-10 and IL-1, play a role in modulating the inflammatory state associated with PCOS (Wujcicka et al., 2017).

This research aimed to study the association between TLR-4 gene polymorphisms and the susceptibility to toxoplasmosis and polycystic ovary syndrome (PCOS) in a sample of infected women. Specifically, the focus was on understanding how variations in the TLR-4 gene may influence the likelihood of developing these conditions, given their significant impact on immune response and inflammation. By examining the genetic differences in women suffering from these diseases, the study sought to uncover potential genetic markers that could predict susceptibility and offer insights into the underlying mechanisms of both toxoplasmosis and PCOS.

Materials and Methods

Study design

A case-control design was employed, involving a total of 150 women, categorized into four distinct groups: 25 samples of females with both polycystic ovary syndrome (PCOS) and toxoplasmosis, 25 samples of females with PCOS without toxoplasmosis, 25 samples of females with toxoplasmosis without PCOS, and a control group consisting of 75 apparently healthy females. This approach allowed for a comprehensive comparison across different combinations of PCOS and toxoplasmosis conditions, facilitating an in-depth analysis of the potential association between TLR4 gene polymorphisms and susceptibility to these conditions.

This study was approved by the Institutional Review Board (I.R.B.) of Al-Nahrain University College of Medicine on November 22, 2021. This study was conducted in accordance with the ethical standards of the Institutional Review Board (IRB) at Al-Nahrain University College of Medicine, which granted approval on November 22, 2021. All procedures involving human participants were performed in compliance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study. Participants were fully informed about the nature, purpose, and potential risks of the study before their inclusion. They were assured that their participation was voluntary and that they could withdraw from the study at any time without any consequences.

Confidentiality and anonymity of the participants were maintained throughout the study. Personal data and medical information were securely stored and accessed only by authorized personnel for research purposes. The findings of this study were reported in aggregate form, ensuring that no individual participant could be identified.

Samples were collected from January 2022 to June 2022 from the Obstetrics and Gynecology Department of Kadhimiya Teaching Hospital in Baghdad province. The participating women were already diagnosed with PCOS and were tested for the presence of anti-T. gondii antibodies using a rapid chromatographic immune technique, which was further confirmed by ELISA. The selected single nucleotide polymorphisms (SNPs) in TLR4 (rs4986791 and rs4986790) were identified using the amplification-refractory mutation system polymerase chain reaction (ARMS PCR) with specific primers.

Diagnostic Criteria

Women participating in the study were previously diagnosed with PCOS based on clinical and biochemical criteria. The presence of Toxoplasma gondii infection was investigated using a rapid chromatographic immune technique and confirmed through the enzyme-linked immunosorbent assay (ELISA).

Genotyping

Genotyping of the selected TLR4 single nucleotide polymorphisms (SNPs), rs4986791 and rs4986790, was performed using the

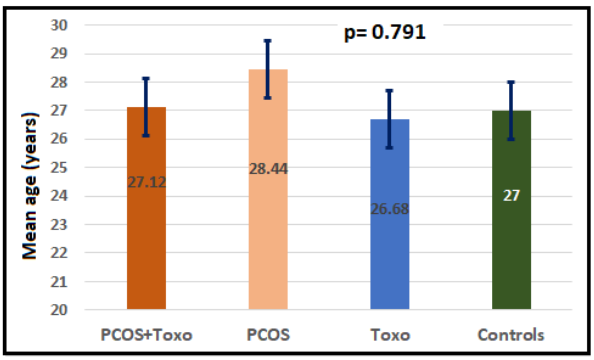

Figure 1. Age distribution of the study population

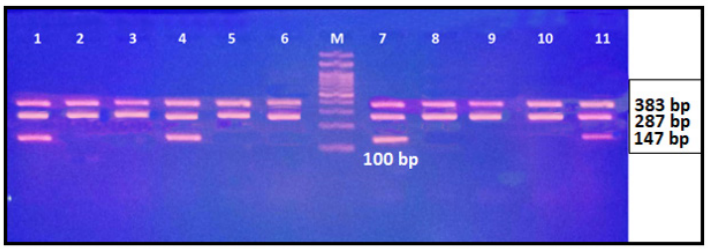

Figure 2. Gel electrophoresis of TLR-4 rs4986790 gene amplified ARMS method. The PCR products were stained with ethidium bromide. Lanes 2,3,5,6,8,9,and 10: AA genotypes; lanes 1,7 and11: AG genotypes. M: molecular marker

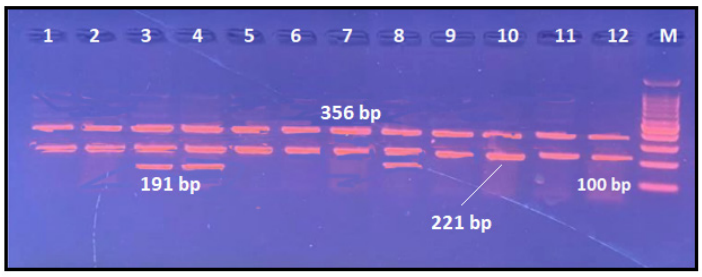

Figure 3. Gel electrophoresis of TLR-4 rs4986791 gene amplified ARMS method. The PCR products were stained with ethidium bromide. Lanes 1,2,5,6,7,9,10,1,and 11: CC genotypes; lanes 3,4 and8: CT genotypes. M: molecular marker

Table 1. Genotypes and alleles TLR-4rs4986790 in patients with PCO+toxoplasmosis and those with PCOS.

rs4986790	PCOS+Tox (n=25)	PCOS (n=25)	P-values	OR(95%CI)
Genotypes				
AA	21(92%)	23(96%)	0.393	1.0
AG	4(8%)	2(4%)		2.19(0.36-13.22)
HWE	0.664	0.677		
Alleles				
A	46(92%)	48(96%)	0.409	1.0
G	4(8%)	2(4%)		2.08(0.37-11.94)

Table 2. Genotypes and alleles TLR-4rs4986790 in patients with PCO+toxoplasmosis and those with toxoplasmosis.

rs4986790	**PCOS+Tox (n=25)**	**Toxoplasma (n=25)**	**P-values**	**OR(95%CI)**
Genotypes				
AA	21(84%)	19(76%)	0.482	1.0
AG	4(16%)	6(24%)		1.66(0.41-6.78)
HWE	0.664	0.820		
Alleles				
A	46(92%)	44(88%)	0.508	1.0
G	4(8%)	6(12%)		1.57(0.41-5.93)

Table 3. Genotypes and alleles TLR-4rs4986790 in patients with PCO+toxoplasmosis and controls.

rs4986790	**PCOS+Tox(n=25)**	**Controls (n=75)**	P-values	OR(95%CI)
Genotypes				
AA	21(84%)	72(96%)	0.058	1.0
AG	4(16%)	3(4%)		4.57(0.95-22.06)
HWE	0.664	0.724		
Alleles				
A	46(92%)	147(98%)	0.064	1.0
G	4(8%)	3(2%)		4.26(0.92-19.74)

Table 4. Genotypes and alleles TLR-4 rs4986790 in patients with PCOS and those with toxoplasmosis.

rs4986790	**PCOS(n=25)**	**Toxoplasma(n=25)**	P-values	OR(95%CI)
Genotypes				
AA	23(96%)	19(76%)	0.140	1.0
AG	2(4%)	6(24%)		3.63(0.66-20.11)
HWE	0.677	0.820		
Alleles				
A	48(96%)	44(88%)	0.159	1.0
G	2(4%)	6(12%)		3.27(0.64-17.07)

Table 5. Genotypes and alleles TLR-4 rs4986790 in patients with PCOS and Control group.

rs4986790	**PCOS(n=25)**	**Controls (n=75)**	P-values	OR(95%CI)
Genotypes				
AA	23(96%)	72(96%)	0.436	1.0
AG	2(4%)	3(4%)		2.09(0.33-13.27)
HWE	0.677	0.724		
Alleles				
A	48(96%)	147(98%)	0.442	1.0
G	2(4%)	3(2%)		2.04(0.331-12.58)

Table 6. Genotypes and alleles TLR-4 rs4986790 in patients with toxoplasmosis and controls

rs4986790	**Toxoplasma (n=25)**	**Controls (n=75)**	P-values	OR(95%CI)
Genotypes				
AA	19(76%)	72(96%)	**0.007**	**1.0**
AG	6(24%)	3(4%)		**7.58(1.73-33.13)**
HWE	0.820	0.724		
Alleles				
A	44(88%)	147(98%)	**0.009**	**1.0**
G	6(12%)	3(2%)		**6.68(1.61-27.82)**

Table 7. Genotypes and alleles TLR-4 rs4986791 in women with PCOS+toxoplasmosis and those with PCOS/or toxoplasmosis alone.

rs4986791	**PCOS+Tox (n=25)**	**PCOS/or Toxo (n=25)**	***P*-value**	**OR(95%CI)**
Genotypes				
CC	23(92%)	24(96%)	0.559	1.0
CT	2(8%)	1(4%)		2.09(0.18-24.62)
HWE	0.834	0.919		
Alleles				
C	48(96%)	49(98%)	0.565	1.0
T	2(4%)	1(2%)		2.04(0.18-23.37)

Table 8. Genotypes and alleles TLR-4 rs4986791 in women with PCOS+toxoplasmosis and those controls.

rs4986791	**PCOS+Tox (n=25)**	**Controls (n=75)**	***P*-value**	**OR(95%CI)**
Genotypes				
CC	23(92%)	74(98.67%)	0.136	1.0
CT	2(8%)	1(1.33%)		6.43(0.56-74.24)
HWE	0.834	0.953		
Alleles				
C	48(96%)	149 (99.33%)	0.140	1.0
T	2(4%)	1(0.67%)		6.21(0.55-69.98)

Table 9. Genotypes and alleles TLR-4 rs4986791 in women with PCOS/ortoxoplasmosis and controls.

rs4986791	**PCOS/or toxo (n=25)**	**Controls (n=75)**	***P*-value**	**OR(95%CI)**
Genotypes				
CC	24(96%)	74(98.67%)	0.432	1.0
CT	1(4%)	1(1.33%)		3.08(0.18-51.2)
HWE	0.919	0.953		
Alleles				
C	49(98%)	149 (99.33%)	0.435	1.0
T	1(2%)	1(0.67%)		3.04(0.18-49.53)

amplification-refractory mutation system (ARMS PCR). This method utilized specific primers designed to detect these SNPs, ensuring accurate identification of the polymorphic variations within the TLR4 gene.

By employing this comprehensive methodology, the study sought to elucidate the potential role of TLR4 gene polymorphisms in influencing susceptibility to PCOS and toxoplasmosis in the study population.

Statistical Analysis

Data were statistically analyzed to compare the frequencies of TLR4 rs4986790 and rs4986791 polymorphisms between the study groups. The analysis aimed to identify any significant associations between these SNPs and the susceptibility to PCOS and toxoplasmosis. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the strength of these associations. Statistical significance was determined using appropriate tests, with a p-value of less than 0.05 considered significant.

Results

Age Distribution of the Study Population

The mean age of women with PCOS and toxoplasmosis was 27.12±6.47 years, which did not significantly differ from the mean ages of women with only PCOS (28.44±5.45 years), women with only toxoplasmosis (28.68±6.41 years), or the control group (27.0±5.56 years).

TLR-4 rs4986790 Polymorphism

The TLR-4 rs4986790 polymorphism was observed in two genotypes: AA and AG across all study groups. The frequencies of these genotypes were similar between women with PCOS and toxoplasmosis, those with only PCOS, and those with only toxoplasmosis, with no significant differences noted. Although the heterozygous genotype (AG) and mutant allele (allele G) were more prevalent among women with both PCOS and toxoplasmosis compared to the control group, this difference was not statistically significant. However, a significant difference was found when comparing women with toxoplasmosis to healthy controls: the AG genotype was more frequent among patients (24%) than controls (4%) with an odds ratio (OR) of 7.58 (95% CI=1.73-33.13, p=0.007). Additionally, the allele G was more common among patients (12%) than controls (2%) with an OR of 6.68 (95% CI=1.61-27.82, p=0.009).

TLR-4 rs4986791 Polymorphism

The TLR-4 rs4986791 polymorphism was also observed in two genotypes: TT and TC across all groups. The frequencies of these genotypes were identical between women with PCOS and those with toxoplasmosis, leading to the merging of these two groups in the analysis. There were no significant differences in the frequencies of genotypes and alleles between all study groups.

Discussion

The current study showed a significant association between the AG genotype of TLR-4 rs4986790 and susceptibility to toxoplasmosis. The AG genotype was more frequent among patients (24%) than controls (4%), with a highly significant difference (OR=7.58, 95% CI=1.73-33.13, p=0.007). Furthermore, the G allele was more common among patients than controls (12% vs. 2%), also showing a highly significant difference (OR=6.68, 95% CI=1.61-27.82, p=0.009). Conversely, no significant association was found between the SNP rs4986791 and any of the included groups.

On a global scale, limited research has been conducted to explore this particular concern. In a study by Wujcicka et al. (2017), a cohort of 116 Polish women—comprising 51 individuals diagnosed with toxoplasmosis and 65 age-matched controls—was examined for potential correlations between four polymorphisms in TLR-2, TLR-4, and TLR-9 and the occurrence of toxoplasmosis. The findings did not reveal any statistically significant associations between the TLR4 rs4986790 or rs3050791 polymorphisms and toxoplasmosis. Conversely, a separate study involving Brazilian children with toxoplasmosis demonstrated an observed relationship between the presence of the C minor allele in TLR9 2848 G>A and the incidence of ocular toxoplasmosis (Wu, 2011).

The precise impact of the rs4986790 SNP on TLR4 structure and/or function remains a topic of ongoing debate within the scientific community. Nevertheless, it is posited that the mutant allele may potentially exert its influence on TLR4 function through any combination of three plausible mechanisms: modulation of TLR4 expression, alteration of TLR4 signaling pathways, or perturbation of TLR4's ability to bind ligands. The prevailing body of research in this field tends to converge on the notion that these SNPs do not exert a substantial effect on the expression levels of TLR4 (Ferwerda et al., 2008; Henckaerts et al., 2007).

In accordance with Wu's 2011 study, it was postulated that an interference occurs within the interaction between the mutant form of TLR4 and serum components such as CD14, LBP, or MD-2, all of which constitute integral elements in the operational response of TLR4. This disruption is attributed to alterations in the receptor's conformation (Ferwerda et al., 2008). In a separate investigation by Henckaerts et al. in 2007, it was suggested that the extracellular domain of the mutant TLR4 possesses a saddle-like surface, with the rs4986790 and rs4986791 amino acids located at opposing ends of this saddle. The concavity situated between these two amino acids implies a potential binding site for either ligands or co-receptors, which could potentially disrupt the receptor's normal functioning (Davoodi et al., 2012).

Undoubtedly, these alterations in conformational structure and interference with ligand docking processes will exert a notable influence on the signaling pathways of the variant TLR4. The investigation conducted by Davoodi et al. in 2012 unveiled

significant disparities in the behavior of NF-kB within mutant TLR4 cells in comparison to their wild-type counterparts when exposed to PAMPs. Moreover, wild-type TLR4 cells exhibited elevated levels of interleukin-1 receptor associated kinase (IRAK), which underwent rapid degradation upon LPS treatment, a phenomenon not observed in mutant TLR4. This observation suggests a dampened signaling cascade and reduced transcription of cytokine genes, as the degradation of IRAK operates as a negative feedback mechanism (Davoodi et al., 2012).

Conclusion

Based on the findings of this study, a significant association was observed between the AG genotype of TLR-4 rs4986790 and susceptibility to toxoplasmosis, with the AG genotype being more prevalent among patients compared to controls. Furthermore, the G allele was found to be more common among patients, indicating a higher susceptibility to toxoplasmosis in individuals carrying this allele. However, no significant association was found between the SNP rs4986791 and any of the included groups. These results suggest that the presence of the G allele within the TLR-4 rs4986790 polymorphism may decrease the interaction between TLR-4 and pathogen-associated molecular patterns from T. gondii, thereby restricting the immune response and increasing susceptibility to infection. This study contributes to our understanding of the genetic factors underlying susceptibility to toxoplasmosis and provides insights into potential mechanisms of immune response modulation mediated by TLR-4 polymorphisms (Zare-Bidaki et al., 2014; Davoodi et al., 2012; Wujcicka et al., 2017).

Author contributions

H.I.F., A.M., H.D., K.E.A.A. developed the Study design, and wrote, reviewed, and edited the paper.

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Competing financial interests

The authors have no conflict of interest.

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