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Association of *TLR2*, *TLR4*, and *TLR7* gene polymorphisms with spontaneous abortion and HCMV serostatus: a case-control study in Iraqi women

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Abstract

Aim: The study aims to explore the relationship between *TLR2* (rs3804100), *TLR4* (rs1927914), and *TLR7* (rs179008) gene polymorphisms and Human *Cytomegalovirus* (HCMV) serostatus in Iraqi women, and to assess the association between spontaneous abortion (SA) and these polymorphisms.

Methods: A case-control study involving 200 women compared 100 who had SAs before 20 weeks of gestation with 100 healthy pregnant controls from Diyala and Babylon Governorates. The study utilised qualitative ELISA to detect HCMV IgG and IgM antibodies in serum and employed the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique for genotyping *TLR2* (rs3804100 T>C), *TLR4* (rs1927914 G>A), and *TLR7* (rs179008 A>T) polymorphisms.

Results: The study revealed that HCMV IgG and IgM antibodies were significantly elevated in women with SA compared to the control group ($P < 0.001$). No notable association was found between the *TLR2* rs3804100 polymorphism and SA. Notably, there were marked differences in the genotype and allele distributions of *TLR4* rs1927914 and *TLR7* rs179008 observed between the cases and controls. Specific genotypes of *TLR4* and *TLR7* genes were associated with modified odds of SA. Furthermore, the high prevalence of HCMV IgG may be linked to genetic associations, particularly TLR genotypes, whereas analysis of HCMV IgM was constrained by the low prevalence observed in control subjects.

Conclusions: Variations in the *TLR4* and *TLR7* genes may be associated with the risk of SA in women in this population. The influence of HCMV seropositivity on immune-related genetic associations should be approached with caution. Further studies with larger sample sizes and consideration of confounding variables are needed.

Keywords

Human *Cytomegalovirus*, PCR-RFLP technique, single-nucleotide polymorphism (SNP), spontaneous abortion, toll-like receptors

Introduction

Spontaneous abortion (SA) refers to the natural termination of a pregnancy prior to the 20th week of gestation and is recognised as one of the most prevalent complications encountered during pregnancy [1, 2]. Ensuring a healthy maternal-fetal immune balance is crucial for a successful pregnancy, as any disruption to this equilibrium heightens the risk of abortion [3, 4].

Toll-like receptors (TLRs) are essential components of innate immunity, as they identify pathogen-associated molecular patterns and initiate suitable immune responses [5]. Throughout pregnancy, these receptors play a crucial role in modulating the immune response within the placenta and maternal tissues, safeguarding against infections while preserving foetal immune tolerance. Prior investigations have indicated that certain TLRs, including *TLR2*, *TLR4*, and *TLR7*, play a role in responses to viral infections and may affect pregnancy outcomes [6–9].

Single-nucleotide polymorphisms (SNPs) are a prevalent form of genetic variation and may affect the effectiveness of the immune response. Current findings indicate that variations in *TLR* genes may influence the immune response to Human *Cytomegalovirus* (HCMV), a prevalent virus that can cause immune disorders during pregnancy. Nevertheless, information concerning the influence of these polymorphisms on the relationship between HCMV and SA is still scarce [10–12].

The HCMV ranks among the most prevalent viruses affecting women of reproductive age [13]. Local studies indicate a significant level of exposure to the virus among pregnant women in Iraq, accompanied by varying antibody levels. Nonetheless, there is a limited number of studies connecting genetic variation in *TLR* to the immune response to HCMV and the risk of SA within the Iraqi population [14–16].

This study sought to assess the connection between genetic variations in the *TLR2*, *TLR4*, and *TLR7* genes and the likelihood of SA. Additionally, it aimed to explore the relationship between these variations and HCMV serostatus (IgG and IgM), thereby addressing an important gap in existing knowledge.

Materials and methods

Study design

This case-control study took place between February 15, 2024, and November 15, 2024, at Al-Batoul Teaching Hospital and Babylon Maternity and Children's Hospital in Iraq. The investigation comprised 200 women, categorised into two distinct groups: 100 women who had undergone SA prior to the 20th week of pregnancy (case group), and 100 healthy pregnant women with no previous history of abortion (control group). Emergency room physicians diagnosed a SA through clinical examination and ultrasound imaging.

Criteria for inclusion and exclusion

The investigation encompassed females aged 20 to 40 years. The criteria for inclusion in the case group comprised a documented history of uninduced SA occurring prior to 20 weeks of gestation, with the cause remaining unidentified. The control group was composed of healthy pregnant women who had no genetic or acquired diseases and no prior history of abortion. Ectopic pregnancies, molar pregnancies, pregnancies associated with diagnosed fibroids, congenital uterine malformations, and abortion s related to autoimmune diseases or cervical insufficiency were excluded.

Ethical approval

The research was approved by the College of Medicine Ethics Committee at the University of Babylon. The collection of samples received approval from the Research and Development Department of the Diyala and

Babylon Health Directorates, in accordance with the Ministry of Health letter No. 8199 dated February 12, 2024. This study complies with the Declaration of Helsinki (2024).

Collection of samples

A total of 5 mL of venous blood was drawn from each participant. The blood samples were collected in tubes containing EDTA for DNA extraction, while serum tubes were utilised for serological testing. The serum was separated by centrifugation, and all samples were stored at –20°C until analysis.

Experimental techniques

Serological identification of HCMV

Qualitative detection of HCMV antibodies, specifically IgG and IgM, was performed by the CMV IgM ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany; Cat. No. CMVM0110) and the CMV IgG ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany; Cat. No. CMVG0110). After ensuring the workspace was properly sterilised and that the kits and other supplies were available. According to the manufacturer's guidelines.

DNA extraction and genotyping study

Genomic DNA was isolated from blood samples using a technique that integrates salt precipitation with silica columns. Primers were custom synthesised by Macrogen Inc. (Seoul, Republic of Korea). PCR amplification was performed using a PCR Master Mix (Cyntol LLC, Moscow, Russia). Restriction enzymes were obtained from SibEnzyme Ltd. (Novosibirsk, Russia). The appropriate restriction enzyme was chosen using the Snap Gene viewer software (V6.0.5).

The DNA was extracted by following the standard protocol [17]. DNA concentration and purity were assessed using a nanodrop device; samples with absorbance ratios (260/280 and 260/230) of 1.8 or higher were selected for further analysis.

Regions of the *TLR2* (rs3804100), *TLR4* (rs1927914), and *TLR7* (rs179008) genes were amplified explicitly through polymerase chain reaction (PCR) as in Table S1. Genomic identification was performed using PCR-restriction fragment length polymorphism (RFLP), as in Figures S1A–C, in which PCR products were digested with the appropriate restriction enzymes for each SNP, according to the manufacturer's guidelines, Table S2 [18]. The digestion products were separated by agarose gel electrophoresis, allowing genotypes to be determined based on the observed band sizes. Tables summarise primer sequences as shown in Table S3. The results of the RCR-RFLP were validated by sequencing individual genes using the Sanger method after selecting three random samples from each gene, each with a genotype at the specified locus, as shown in Figures S2, S3, and S4.

Data evaluation

Statistical analysis was conducted utilising SPSS version 28. The comparison of continuous variables between the two groups was performed using an independent *t*-test based on the distribution characteristics. Genotype distributions and allelic frequencies were examined through the application of the *chi*-squared test or Fisher's test, as deemed suitable. The association between genotypes and the risk of abortion was evaluated using logistic regression, with odds ratios (ORs) and 95% confidence intervals calculated. The *chi*-squared test was used to assess the Hardy–Weinberg equilibrium (HWE) exclusively in the control group. *P* values below 0.05 were deemed statistically significant.

Results

Baseline clinical and demographic characteristics of study participants

The *chi*-square results show a significant difference ($P = 0.016$) in the frequency of SA between the 31–40 age group and the younger age groups. The *chi*-square and logistic regression analyses showed a significant difference (OR = 14.333, $P < 0.001$), a 14-fold higher risk of SA in the first trimester, and a higher prevalence in later trimesters Table 1.

Table 1. Demographic data for the studied groups.

Variables		Groups		Measures	
		Abortion (*n*, %)	Pregnant (*n*, %)	*P*-value	OR (95% CI)
Age groups	20–30 yr	44 (44.0%)	61 (61.0%)	0.099	0.721 (0.490–1.063)
	31–40 yr	56 (56.0%)	39 (39.0%)	0.083	1.436 (0.954–2.161)
	X^2 = 5.794 *P* = 0.016*				
Trimester	First trimester	86 (86.0%)	6 (6.0%)	0.001*	14.333 (6.265–32.792)
	Second trimester	14 (14.0%)	18 (18.0%)	0.481	0.778 (0.387–1.564)
	Third trimester	0 (0.0%)	76 (76.0%)	-	-
	X^2 = 146.065 *P*-value < 0.001*				

* Significant differences at *P*-value < 0.05.

Prevalence of HCMV IgG and IgM Antibodies

The results of the logistic regression and *chi*-square analyses demonstrated a statistically significant presence of HCMV IgM and IgG in case and control groups (*P*-value < 0.05). The findings indicate that HCMV IgM is linked to a risk factor of (OR = 22.000), and HCMV IgM is observed four times more frequently in women who experienced abortion compared to the control group (Table 2).

Table 2. Prevalence of HCMV IgM and IgG antibodies in abortion and pregnant groups.

Variables		Groups		Measures		
		Abortion (*n*, %)	Pregnant (*n*, %)	*P*-value	OR	95% CI
HCMV IgM	Positive	22 (22.0%)	1 (1.0%)	0.003*	22.000	2.965–163.213
	Negative	78 (78.0%)	99 (99.0%)	0.115	0.788	0.586–1.060
	X^2 = 21.665 *P* < 0.001*					
HCMV IgG	Positive	93 (93.0%)	20 (20.0%)	0.001*	4.650	2.868–7.538
	Negative	7 (7.0%)	80 (80.0%)	0.001*	0.088	0.040–0.189
	X^2 = 108.412 *P* < 0.001*					

* Significant differences at *P*-value < 0.05.

Prevalence of investigated *TLR2-SNP* genotype in aborted and pregnant women

The HWE equation was employed to investigate the distribution of *TLR2-SNP* genotypes, as in Figure S1A. The results indicated that the frequency of the reference allele (T) was 77% in the abortion group and 78% in the pregnant group, whereas the (C) allele was 23% and 22%, respectively. Nevertheless, no statistically significant variations were seen in either allelic or genotypic frequencies between the two groups. Statistical analyses employing codominant (TC and CC vs. TT), dominant (TC and CC vs. TT), recessive, and over-dominant genetic models for the (TC vs. TT and CC) genotypes, as illustrated in Table 3.

Table 3. Genotyping and allelic frequency association of polymorphism of the TLR2 gene.

(rs3804100) Locus	Abortion			Pregnant		X^2	*P* (HWE)	OR (95% CI)	*P*- value
	TLR2	*n*	%	*n*	%				
Alleles	T	153	77%	156	78%	0.128	0.720	Ref.	
	C	47	23%	44	22%			1.089	0.721
	Total	200	100%	200	100%			(0.682–1.739)	

Table 3. Genotyping and allelic frequency association of polymorphism of the TLR2 gene. (*continued*)

(rs3804100) Locus	Abortion			Pregnant		X^2	*P* (HWE)	OR (95% CI)	*P*- value
	TLR2	*n*	%	*n*	%				
Codominant	TT	57	57%	63	63%	2.292	0.318	Ref.	
	TC	39	39%	30	30%			1.437 (0.792–2.607)	0.233
	CC	4	4%	7	7%			0.632 (0.176–2.271)	0.482
	Total	100	100%	100	100%				
Dominant	TT	57	57%	63	63%	0.75	0.386	Ref.	
	TC-CC	43	43%	37	37%			1.284 (0.729–2.265)	0.387
	Total	100	100%	100	100%				
Recessive	TT-TC	96	96%	93	93%	0.866	0.352	Ref.	
	CC	4	4%	7	7%			0.554 (0.157–1.954)	0.358
	Total	100	100%	100	100%				
Overdominant	TT-CC	61	61%	70	70%	1.792	0.181	Ref.	
	TC	39	39%	30	30%			1.492 (0.83–2.683)	0.182

HWE: Hardy–Weinberg equilibrium.

Relevance of *TLR4* (rs1927914) genotypic and allelic frequencies

The correlation between the *TLR4* gene polymorphism rs1927914 (chr9:117702447, G>A) and the SA group was assessed using several genetic inheritance models, as shown in Figure S1B. The co-dominant model demonstrated a statistically significant difference between the GA and GG genotypes compared to the AA reference genotype (OR = 0.483; $P_{(HWE)}$ = 0.026; P = 0.021). The over-dominant model comparing GA to (AA and GG) genotypes demonstrated a significant link (OR = 0.463; $P_{(HWE)}$ = 0.007; P = 0.008). Neither the dominant nor the recessive models exhibited substantial differences between the abortion and control groups ($P > 0.05$), as illustrated in Table 4.

Table 4. Allelic frequency and genotyping frequency association of the *TLR4* Gene.

(rs1927914) Locus G>A	TLR4	Abortion (*n*, %)	Pregnant (*n*, %)	X^2	$P_{(HWE)}$	OR (95% CI)	*P*- value
Alleles	A	123 (61.5%)	118 (59%)	0.261	0.609	Ref.	
	G	77 (38.5%)	82 (41%)			0.901 (0.603–1.345)	0.610
	Total	200 (100%)	200 (100%)				
Codominant	AA	41 (41%)	29 (29%)	7.321	0.026*	Ref.	
	GA	41 (41%)	60 (60%)			0.483 (0.26–0.898)	0.021*
	GG	18 (18%)	11 (11%)			1.157 (0.476–2.813)	0.747
	Total	100 (100%)	100 (100%)				
Dominant	AA	41 (41%)	29 (29%)	3.165	0.075	Ref.	
	GA-GG	59 (59%)	71 (71%)			0.588 (0.327–1.058)	0.076
	Total	100 (100%)	100 (100%)				
Recessive	AA-GA	82 (82%)	89 (89%)	1.976	0.160	Ref.	
	GG	18 (18%)	11 (11%)			1.776 (0.792–3.984)	0.163
	Total	100 (100%)	100 (100%)				
Over-dominant	AA-GG	59 (59%)	40 (40%)	7.221	0.007*	Ref.	
	GA	41 (41%)	60 (60%)			0.463 (0.263–0.815)	0.008*
	Total	100 (100%)	100 (100%)				

* Significant differences at *P*-value < 0.05. $P_{(HWE)}$: *P*-value of Hardy–Weinberg equilibrium.

Genotypic and allelic examination of *TLR7* gene rs179008 A>T SNP

Chi-square analysis of the allelic and genotypic frequencies of the *TLR7* gene revealed statistical significance for the T allele (OR = 0.644, $P_{(HWE)}$ = 0.047, *P* = 0.048) in healthy pregnant women when compared to the reference A allele (Figure S1C). A statistically significant difference in genotype frequency distribution was detected in the codominant mode (AT and TT compared to AA) among healthy pregnant women with a reduced risk of SA (OR = 0.217, *P*-value = 0.001). Similarly, the dominant mode (AT + TT vs. AA) exhibited an OR of 0.357 with a $P_{(HWE)}$ value of = 0.001, and the over-dominant mode (AT vs. AA + TT) demonstrated an OR of 0.213 with a $P_{(HWE)}$ value of < 0.001 in healthy pregnant women compared to the reference genotype AA (Table 5).

Table 5. Genotyping frequency and allelic frequency association of the *TLR7* Gene.

(rs179008) Locus A>T	*TLR7*	Abortion (*n*, %)	Pregnant (*n*, %)	X^2	$P_{(HWE)}$	OR (95% CI)	*P*- value
Alleles	A	151 (75.5%)	133 (66.5%)	3.934	0.047*	Ref.	
	T	49 (24.5%)	67 (33.5%)			0.644	0.048*
	Total	200 (100%)	200 (100%)			(0.417–0.996)	
Codominant	AA	67 (67%)	42 (42%)	23.209	0.001*	Ref.	
	AT	17 (17%)	49 (49%)			0.217	0.001*
						(0.111–0.426)	
	TT	16 (16%)	9 (9%)			1.114	0.814
	Total	100 (100%)	100 (100%)			(0.452–2.75)	
Dominant	AA	67 (67%)	42 (42%)	12.602	0.001*	Ref.	
	AT + TT	33 (33%)	58 (58%)			0.357	< 0.001*
	Total	100 (100%)	100 (100%)			(0.201–0.634)	
Recessive	AA + AT	84 (84%)	91 (91%)	2.240	0.134	Ref.	
	TT	16 (16%)	9 (9%)			1.926	0.139
	Total	100 (100%)	100 (100%)			(0.808–4.592)	
Over-dominant	AA + TT	83 (83%)	51 (51%)	23.157	< 0.001*	Ref.	
	AT	17 (17%)	49 (49%)			0.213	< 0.001*
	Total	100 (100%)	100 (100%)			(0.111–0.409)	

* Significant differences at *P*-value < 0.05. $P_{(HWE)}$: *P*-value of Hardy–Weinberg equilibrium.

Relation of *TLRs* Polymorphisms with HCMV IgG and IgM in pregnant and aborted women

TLRs polymorphisms associated with HCMV IgG in the studied group

Logistic regression and chi-square analysis found strong links between HCMV IgG positivity and the *TLR2* TT and TC genotypes. Women with the TT and TC genotypes showed a 5.600-fold and 4.250-fold increased risk of abortion compared to healthy pregnant women. Similarly, all *TLR4* rs1927914 genotypes were substantially linked with abortion risk, including AA (OR = 5.571, *P* = 0.0001), GA (OR = 4.100, *P* = 0.0001), and GG (OR = 4.333, *P* = 0.022). For *TLR7* rs179008, the TT genotype was significantly linked with abortion (OR = 13.000, 95% CI: 1.701–99.375, *P* = 0.013), indicating a 13-fold increased risk of abortion compared to the pregnant control group (Table 6).

Table 6. Comparison of *TLR* genes, genotype, and presence of HCMV IgG in the two groups.

HCMV IgG		Groups		Measures	
		Abortion (*n*, %)	Pregnant (*n*, %)	*P*-value	OR (95% CI)
TLR2	TT	56 (60.2%)	10 (50.0%)	0.0001*	5.600 (2.857–10.975)
	TC	34 (36.6%)	8 (40.0%)	0.0001*	4.250 (1.967–9.181)
	CC	3 (3.2%)	2 (10.0%)	0.657	1.500(0.251–8.977)
	Total	93 (100%)	20 (100%)	-	
	X^2 = 2.054 *P* < 0.342				

Table 6. Comparison of *TLR* genes, genotype, and presence of HCMV IgG in the two groups. (*continued*)

HCMV IgG		Groups		Measures	
		Abortion (*n*, %)	**Pregnant (*n*, %)**	***P*-value**	**OR (95% CI)**
TLR4	AA	39 (41.9%)	7 (35.0%)	0.0001*	5.571 (2.492–12.456)
	GA	41 (44.1%)	10 (50.0%)	0.0001*	4.100 (2.054–8.185)
	GG	13 (14.0%)	3 (15.0%)	0.022*	4.333 (1.235–15.206)
	Total	93 (100%)	20 (100%)	-	
	X^2 = 0.334 P < 0.894				
TLR7	AA	62 (66.7%)	10 (50.0%)	0.0001*	6.200 (3.179–12.091)
	AT	18 (19.4%)	9 (45.0%)	0.090	2.000 (0.899–4.452)
	TT	13 (14.0%)	1 (5.0%)	0.013*	13.000 (1.701–99.375)
	Total	93 (100%)	20 (100%)	-	
	X^2 = 6.319 P < 0.044*				

* Significant differences at *P*-value < 0.05.

Identification of HCMV IgM and *TLR2*, *TLR4*, and *TLR7* polymorphisms genes

Statistical analysis utilising chi-square and logistic regression revealed a statistically significant association (OR = 11.000, P = 0.022) concerning *TLR4* gene polymorphism, indicating that women with genotype AA were 11 times more predisposed to SA compared to other genotypes among seropositive HCMV IgM in the aborted women, as illustrated in Table 7.

Table 7. Comparison of *TLR* genotypes and presence of HCMV IgM in the two groups.

HCMV IgM		Groups		Measures	
		Abortion (*n*, %)	**Pregnant (*n*, %)**	***P*-value**	**OR (95% CI)**
TLR2	TT	13 (59.1%)	0 (0.0%)	0.996	-
	TC	7 (31.8%)	1 (100.0%)	0.069	7.000 (0.861–56.895)
	CC	2 (9.1%)	0 (0.0%)	0.657	-
	Total	22 (100%)	1 (100%)	-	
	X^2 = 2.594 P < 0.435				
TLR4	AA	11 (50.0%)	1 (100.0%)	0.022*	11.000 (1.420–85.201)
	GA	9 (40.9%)	0 (0.0%)	0.997	-
	GG	2 (9.1%)	0 (0.0%)	-	-
	Total	22 (100%)	1 (100%)	-	
	X^2 = 1.746 P < 1.000				
TLR7	AA	15 (68.2%)	0 (0.0%)	0.997	-
	AT	3 (13.6%)	1 (100.0%)	0.341	3.000 (0.312–28.841)
	TT	4 (18.2%)	0 (0.0%)	-	Not estimable
	Total	22 (100%)	1 (100%)	-	
	X^2 = 3.837 P < 0.348				

* Significant differences at *P*-value < 0.05. For certain genotypes, zero cell counts made it impossible to determine odds ratios with any degree of accuracy.

Discussion

This study investigated the association between genetic polymorphisms in the *TLR2*, *TLR4*, and *TLR7* genes with the risk of SA, highlighting the impact of *Cytomegalovirus* serostatus on the immunological response during pregnancy. The results arise amid increasing focus on the significance of innate immunity and genetic variation in pregnancy-related diseases.

Women aged 20–40 were selected for the current study because they are the most active reproductive age group. According to this study, the age group (31–40 years old) is 56.0% younger than the abortion group. According to the current findings, SA happens at a higher rate in the first trimester (86.0%) than in normal pregnancies (6.0%), confirming that this time is the riskiest. This data is comparable with research

[19], which found that more than 80% of SAs occur before the 12th week of pregnancy, especially in older women or those with a history of abortion Table 1.

HCMV is the most prevalent congenital infection globally, affecting 0.7% to 1% of live births. While 11% exhibit symptoms and 30% to 40% experience developmental delays, routine screening for infections in the womb is not recommended due to the risk of misinterpreting serological data. IgG antibodies indicate past exposure, but variability in prevalence across populations complicates understanding [20, 21]. Additionally, factors such as other illnesses and immune status can affect outcomes, and serological and molecular diagnostics differ significantly [22].

Pattern recognition receptors, particularly *TLR2*, play a vital role in immune response activation and the pathogenesis of infectious diseases. However, research on the link between *TLR* gene polymorphisms and SA remains inconclusive. Studies by Kuliczkowska-Płaksej et al. [23] and Semlali et al. [24] found no significant genetic differences between affected and control groups in women with polycystic ovarian syndrome and breast cancer patients, respectively. In contrast, Ying et al. [25] reported increased levels of TLR2 and TLR4 proteins and mRNA in decidua tissue from women with recurrent abortion s, which correlated with higher Th1 cytokine levels. This study found no association between the *TLR2* (rs3804100) polymorphism and SA in women (Table 3).

The *TLR4* gene (rs1927914) analysis revealed significant differences in genotype prevalence between the SA and control groups, particularly for the conjugate and predominant genotypes in Table 4. The heterozygous (GA) genotype was associated with a lower OR for abortion, indicating a potential heterozygote advantage that enhances immunological balance during pregnancy by mitigating excessive inflammation while maintaining immune defenses. A significant association was found between the *TLR4* rs1927914 polymorphism and the risk of SA, with the G allele more prevalent among healthy pregnant women and protective effects in heterozygous GA and GG genotypes (OR = 0.483 and 0.463, $P < 0.05$). These findings contrast with Jiao et al. [26], who identified rs1927914 as a genetic marker for recurrent abortion in various populations. Altered *TLR4* expression has also been linked to increased abortion frequency, while the GA genotype's association with reduced incidence suggests its role in sustaining a balanced inflammatory response during pregnancy [27].

The investigation reveals a significant association between the *TLR7* gene polymorphism (rs179008, A>T) and a lower risk of abortion without external assistance (Table 5). The T allele is more common in healthy pregnant women, indicating a possible protective effect. The heterozygous (AT vs. AA-TT) genotype shows a marked reduction in abortion risk under both codominant and dominant models, suggesting a heterozygote advantage due to balanced *TLR7*-mediated immune regulation during pregnancy. The TT genotype did not show a significant correlation, implying the protective advantage might be limited to heterozygotes or influenced by other factors. The rs179008 SNP results in an amino acid substitution in the TLR7 protein, potentially affecting immune tolerance required for pregnancy [28].

The study in Table 6 found a higher prevalence of HCMV IgG positivity among women who experienced abortions compared to a control group, indicating prior exposure to the virus. However, HCMV IgG levels should not be viewed as a sign of active infection but rather as reflecting immunological factors that may influence the correlation between *TLR* receptor genetic diversity and the immune response during pregnancy. The analysis of HCMV IgM in Table 7 was complicated by a low number of positive cases in the control group. The findings revealed that women with TT and TC genotypes of the *TLR2* gene exhibit a significantly increased abortion risk, in line with earlier studies on Iraqi women. *TLR4* polymorphisms (rs1927914) were also linked to heightened abortion risk in HCMV IgG-positive women, suggesting that *TLR4* variants may affect immune responses to infections. Additionally, women with the AA genotype of *TLR7* and positive HCMV IgG status have about a sixfold greater risk of abortion, indicating the importance of *TLR7* polymorphisms in virus-related pregnancy loss. Previous research supports an association between HCMV IgG levels and adverse pregnancy outcomes, highlighting the need for further investigation of TLR polymorphisms and HCMV antibodies during pregnancy [29–31].

This study found no strong link between *TLR2* genotypes and abortion outcomes but noted that HCMV infection can disrupt gene expression and placental function. The absence of *TLR2* in syncytiotrophoblasts may reduce immunological activation in early pregnancy. HCMV IgM positivity is correlated with a higher abortion likelihood in those with the *TLR4* AA genotype. Understanding TLR-mediated viral resistance is essential, as TLR polymorphisms impact immune responses to HCMV. *TLR4* activation enhances IgM production, which influences defenses against HCMV. Previous research has identified associations between *TLR4* polymorphisms and infection susceptibility, highlighting TLRs' role in immune control. While HCMV IgM positivity is more common in women with abortion histories, no significant link was found between *TLR7* genotypes and abortion risk [13, 32, 33].

The combined study of *TLR* gene variants and HCMV serostatus suggests that the interaction between genetic and environmental/viral factors may be more significant than the effects of each factor alone. This interaction may elucidate the variety noted in the outcomes of previous investigations involving heterogeneous populations, including disparities in genetic backgrounds, viral exposure patterns, and environmental influences.

This study has limitations, including a small sample size in some sub-analyses of the HCMV IgM-positive group and a difference in gestational age between the case and control groups. Also, the PCR-RFLP approach is reliable but may not be as accurate as newer genetic methods. This work is notable as one of the first investigations in Iraq evaluating the correlation between genetic diversity in *TLR* genes and HCMV infection in connection to SA, hence providing significant exploratory scientific value.

In conclusion, this study's findings suggest that the rs1927914 genetic polymorphism in the *TLR4* gene, particularly the heterozygous (GA) genotype, may be associated with a reduced risk of SA. The rs179008 polymorphism in the *TLR7* gene may affect the immunological response during pregnancy. The findings suggest that HCMV serostatus may affect the relationship between certain *TLR* gene polymorphisms and pregnancy, although a direct causative relationship has yet to be confirmed. However, further studies with larger sample sizes and more stringent techniques are needed to confirm these findings and elucidate the underlying mechanisms.

Abbreviations

HCMV: Human *Cytomegalovirus*

HWE: Hardy–Weinberg equilibrium

ORs: odds ratios

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism

SA: spontaneous abortion

SNP: single-nucleotide polymorphism

TLRs: toll-like receptors

Supplementary materials

The supplementary material for this article is available at: https://www.explorationpub.com/uploads/Article/file/1003258_sup_1.pdf.

The supplementary material for this article is available at: https://www.explorationpub.com/uploads/Article/file/1003258_sup_2.pdf.

Declarations

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Author contributions

NMK: Conceptualization, Investigation, Writing—original draft, Writing—review & editing, Visualization, Formal analysis. BJAT: Validation, Writing—review & editing, Supervision. AKG: Validation, Writing—review & editing, Supervision. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The research was approved by the College of Medicine Ethics Committee at the University of Babylon. The collection of samples received approval from the Research and Development Department of the Diyala and Babylon Health Directorates, in accordance with the Ministry of Health letter No. 8199, dated February 12, 2024. This study complies with the Declaration of Helsinki (2024).

Consent to participate

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Not applicable.

Availability of data and materials

The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

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