Original Article

Association between Genetic Variation of Interleukin-6 with Cytomegalovirus Infection in Women Having Recurrent Miscarriages

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Abstract

Background: Recurrent miscarriage is characterized by two or more spontaneous abortions and can be attributed to various factors, including anatomy, endocrinology, genetics, immunology, and microbial infections, although the exact cause remains unclear in up to 50% of cases, with the genetic background of the host, cytomegalovirus (CMV) infections, and interleukin-6 (IL-6) single nucleotide polymorphisms being of significant importance. **Objectives:** Particular primers were used to study single nucleotide polymorphisms (-174 r1800795 G/C) in the IL-6 gene in order to assess the correlation between CMV infection and recurrent pregnancy loss (RPL) vulnerability through the employment of tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). **Materials and Methods:** Fifty female subjects diagnosed with recurrent pregnancy loss and cytomegalovirus infection were selected as the case group, whereas the control group consisted of 50 healthy individuals who had given birth without CMV infection; blood samples were collected from September 2022 to January 2023. DNA was extracted to study single nucleotide polymorphisms in the IL-6 gene. **Results:** The PCR analysis yielded results that indicate the presence of G and C alleles, with three distinct genotypes (GG, GC, and CC) and varying lengths (302, 206, and 152) bp. Additionally, the stratified analysis revealed a strong and significant association between the patient and control groups. **Conclusion:** Potential association between the rs1800795 G>C polymorphism in IL-6 gene and RPL susceptibility.

Keywords: CMV, genetic susceptibility, interleukin-6, recurrent miscarriage, tetra ARMS-PCR

INTRODUCTION

Recurrent miscarriage (RM) is a medical condition characterized by the occurrence of two or more pregnancy losses prior to the 20th week following the last menstrual cycle. This condition affects an estimated 1%-3% of couples and is commonly referred to as recurrent pregnancy loss or recurrent miscarriage.[1] Approximately 50% of cases of recurrent pregnancy loss (RPL) have an unknown etiology, despite various causal factors such as chromosomal abnormalities in parents, uterine abnormalities, infectious illnesses, endocrine abnormalities, and autoimmune defects being associated with RM.[2] Viral causes are responsible for the majority of congenital infections that pose a substantial threat to fetal well-being. The human uterus is often infected by Rubella, cytomegalovirus (CMV), and toxoplasma, resulting in congenital anomalies, stillbirth, abortion, and premature infants.[3]

Human cytomegalovirus (HCMV), otherwise referred to as human herpesvirus 5, serves as the archetypal member of the *Betaherpesvirinae* family. As with all herpesviruses, it is capable of establishing latency and consequently endures for the individual's lifetime. HCMV infection is prevalent worldwide.[4] The prevalence of congenital HCMV infection is high. The clinical presentation of

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HCMV infection can range from an absence of symptoms to severe symptomatic manifestations. The latter may be characterized by petechiae, microcephaly, chorioretinitis, hepatosplenomegaly, and growth retardation.[5,6] During pregnancy, the onset of congenital cytomegalovirus may ensue due to the reactivation of previously acquired cytomegalovirus or primary infection.[7] Some investigations have exhibited the significance of CMV-related infection in the unmanaged loss of pregnancy; studies have reported that infections caused by cytomegalovirus are a possible causative agent in inducing pregnancy loss in women.[8] nevertheless, the implications of CMV infection on RPL remain indeterminate.

However, it has been suggested that immunogenetic variables have a significant contribution to the etiopathogenesis of recurrent miscarriage. The maintenance of a successful pregnancy has been associated with the balance of Th1 and Th2 cytokines.[9] Additionally, the physiological growth of the human fetus during gestation is linked to the decrease in Th1 cytokines, including IL-6, tumor necrosis factor (TNF)-α, and IL-10.[10] A prolonged exposure to Th1 cytokines can stimulate a cell-mediated immune response that is harmful to the fetus, leading to pregnancy losses.[11] The expression of cytokines in humans is controlled by their genetic constitution, thereby indicating the effect of polymorphisms on the rate of cytokine production.[12,13]

IL-6 plays a pivotal role in the regulation of inflammation and exerts both pro-inflammatory and anti-inflammatory effects on the immune system.[14] Within the IL-6 gene region, a plethora of single nucleotide polymorphisms (SNPs) exist that can modulate IL-6 expression, thereby influencing downstream target genes. Prior investigations have posited a plausible association between -634C/G and -174 G/C SNPs and modified IL-6 expression, as well as an augmented risk of RPL.[15] Nevertheless, certain inquiries have evinced a negative correlation between IL-6 polymorphism and the risk of RPL. The goal of this study is to analyze the connections between IL-6 gene variation G/C at position (174), CMV infection in females with RM.

Materials and Methods

Patients and sample collection

The study employed a case-control design and enlisted 50 female subjects diagnosed with RPL and CMV infection as the case group, whereas the control group was composed of 50 healthy individuals who had successfully given birth without CMV infection. The cases were female subjects aged between 16 and 45 years who had experienced at least two consecutive abortions before the 20th week of gestation. The samples were procured between September 2022 and January 2023 from Imam Al-Sadiq Hospital and Babel Teaching Hospital for Women and Children, post obtaining informed consent from all participants. 2.5 milliliters of blood samples were collected in anticoagulant tubes from each participant, with the aim of extracting DNA for subsequent analysis.

Extraction of DNA

The collected blood samples were subjected to DNA extraction as per the guidelines provided by the kit manufacturer (Favorgen, Pingtung City, Taiwan).[16]

Primer design and genotyping

The current investigation utilized tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) polymorphism to conduct the genotyping of IL-6 polymorphism − 174 G>C [rs1800795]. For this purpose, specific primers were designed and the amplification was conducted in a thermal cycler with a reaction mixture volume of 25 μL. The reaction mixture comprised of 2 μL genomic DNA, 1.5 μL forward primer, and 1.5 μL reverse primer for each inner and outer primers [Table 1], 12 μL Green Master Mix (Promega, Madison, Wisconsin, United States), and 8 μL free nuclease water. The reaction conditions were mentioned in Table 2. Electrophoresis was used to separate the amplified fragments on a 1% agarose gel that had been stained with ethidium bromide.

Ethical approval

All patients granted verbal consent, thereby securing ethical approval for the study. The publication ethics committee at the Women's College of Science, University of Babylon, Iraq, has authorized this research under the reference number (4)/M.Sc dated on August 3, 2023.

Statistical analysis

The acquired data were statistically analyzed using the Statistical Package for Social Sciences (version 28; SPSS Inc., Chicago, Illinois) software in the present investigation. The team of researchers employed the Chi-square test method to determine the common IL-6 polymorphism and CMV roles in RPL. The Chi-square test was utilized

Table 1: Primers used for tetra ARMS-PCR

Name	Sequence 5′ to 3′	Length bp
IL-6 forward outer primer	GACATGCCAAAGTGCTGAGTCACTAA	302
IL-6 reverse outer primer	GAATGAGCCTCAGACATCTCCAGTCCTA	302
IL-6 forward inner primer [G allele]	GCACTTTTCCCCCCTAGTTGTGTCTTCCG	206
IL-6 reverse inner primer [C allele]	ATTGTGCAATGTGACGTCCTTTAGCTTG	152

ARMS-PCR: amplification refractory mutation system-polymerase chain reaction to determine the odds ratio (OR) value along with the 95% confidence interval (CI). The statistical significance level was deemed established when the *P*-values were below 0.05. Furthermore, the projected prevalence of genotype was calculated by utilizing the allele frequencies in accordance with the Hardy-Weinberg equilibrium.

RESULTS

Genotypes of IL-6 − 174 G>C (rs1800795)

In this study, the PCR analysis yielded results that indicate the presence of G and C alleles, with three distinct genotypes (GG, GC, and CC) and varying lengths (302, 206, and 152) bp, as illustrated in Figures 1 and 2. The frequencies of the rs1800795 G>C polymorphism were found to be 46% and 24% for the homozygous genotypes GG and CC, respectively, in the patient group, and 30% for the heterozygous genotype GC. The healthy control group, on the other hand, exhibited polymorphic alleles GG, GC, and CC at frequencies of 80%, 6%, and 14%, respectively, as presented in Table 3. These findings suggest a potential association between the rs1800795 G>C polymorphism and disease susceptibility. Additionally, the stratified analysis revealed a strong and significant association between the patient and control groups.

Table 2 : Conditions of tetra ARMS-PCR for IL-6 genotyping

Step	Cycle	Temperature (°C)	Time
Initial denaturation	1	95	3 min
Denaturation	30	95	40 s
Annealing		61	40 s
Extension		72	40 s
Final extension	1	72	3 min

ARMS-PCR: amplification refractory mutation system-polymerase chain reaction

DISCUSSION

Recurrent miscarriage is a prevalent affliction that afflicts women, and it poses a significant issue for reproductive health as it affects roughly one in 300 pregnancies.[17] The multifaceted nature of recurrent miscarriage requires an investigation into various factors to determine the underlying causes of this condition.

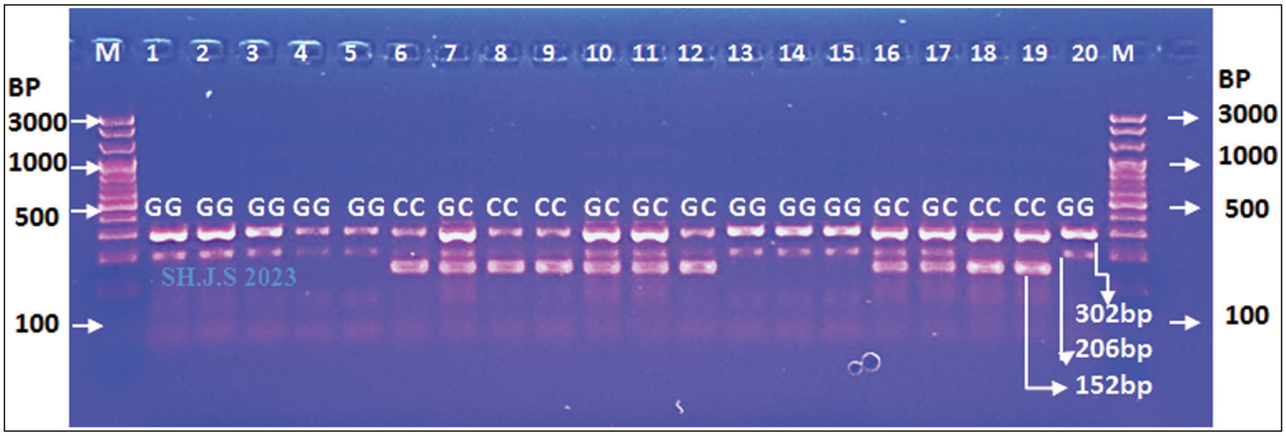

Figure 1: Electrophoresis for PCR product of IL-6 gene for cases. Lane (7, 10, 11, 12, 16, 17) heterozygote GC result (206 bp). Lane (1, 2, 3, 4, 5, 13, 14, 15, 20) homozygotes GG result (302 bp) and lane (6, 8, 9, 18, 19) homozygotes CC result (152 bp). 1% agarose, TBE buffer, pre-stained by ethidium bromide. Time 45 min/100 volt

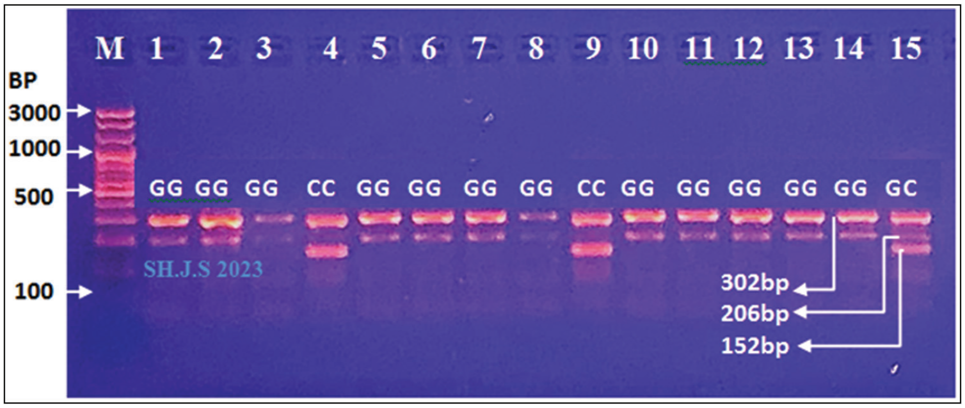

Figure 2: Electrophoresis for PCR product of IL-6 gene for control group. Lane (15) heterozygote GC result (206 bp). Lane (1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 14) homozygotes GG result (302 bp) and lane (4, 9) homozygotes CC result (152 bp). 1% agarose, TBE buffer, pre-stained by ethidium bromide. Time 45 min/100 volt

Table 3: Genotypes and allele frequencies of IL-6 − 174 G>C (rs1800795)

Model	genotype	Case (50)		Control (50)		*P*-value	OR (95% CI)
		No.	%	No.	%	Reference	
Codominant	GG	23	46	40	80		
	GC	15	30	3	6	<0.001*	0.11 (0.03-0.44)
	CC	12	24	7	14	0.04*	0.33 (0.11-0.97)
Dominant	GG	23	46	40	80	Reference	
	GC+CC	27	54	10	20	<0.001*	0.21 (0.08-0.51)
Recessive	CC	12	24	7	14	Reference	
	GC+GG	38	64	43	86	0.25	1.80 (0.64-5.06)
Over-dominant	GC	15	30	3	6	Reference	
	GG+CC	35	70	47	94	0.002*	6.71 (1.80-24.99)
Alleles	G	61	61	83	83	Reference	
	C	39	39	17	17	<0.001*	0.32 (0.16-0.61)

*Significant statistically $P < 0.05$

CI: confidence interval, OR: odd ratio

These factors encompass coagulation factors, infection factors, immunological factors, anatomical issues, and chromosomal abnormalities.[18] Some genetic-related factors, such as genetic polymorphisms, have been linked to poor pregnancy outcomes, including the occurrence of RPL. Research has suggested that cytokines, which regulate immune responses, play a crucial role in maintaining a healthy pregnancy. Therefore, it is important to understand the genetic and immunological factors that contribute to RPL in order to develop effective strategies for its prevention and management.[19] The presence of IL-6 and IL-10, which are anti-inflammatory cytokines, is considered crucial for maintaining a healthy gestation period and acting as the primary defense mechanism against viral infections. Functional SNPs with impact on IL-6 expression are crucial in the pathogenesis of recurrent pregnancy loss at various stages. The significant outcome of the research indicated a positive correlation between IL-6 SNP and the risk of RPL. The IL-6-174C/G polymorphism is situated 174 base pairs upstream of the promoter of the IL-6 gene, which may regulate IL-6 transcription or posttranscriptional modification. Genetic predisposition may have an impact on the probability of, susceptibility to, or chronicity of CMV infection, along with the pace of RPL progress.[20] Several research studies have been conducted to explore the association between different cytokine polymorphisms and RPL. In addition, several other studies and investigations have not been successful in identifying a link between the common polymorphisms in the IL-6 SNP and the risk of RPL.[21] Our findings demonstrate a significant correlation between the risk of recurrent pregnancy loss and the presence of the C allele in the IL-6 -174 gene among the cases under study. In contrast to the findings of Ardakani *et al.*,[3] it has been observed that there exists no association between the risk of RPL and IL-6 -174 SNP. The observed discrepancies in the outcomes of the aforementioned studies may have been influenced by ethnic variances or the influence of patients' enrollment criteria. IL-6, a type of cytokine, has been shown to play a protective role in the immune system's response to bacterial and viral infections. Pathogenic agents must penetrate the fetal tissues or endometrium's intrauterine environment to induce an inflammatory response, which may culminate in a miscarriage.[22] In the present investigation, our findings have demonstrated a noteworthy correlation between RPL and patients who are positive for CMV with allele C in IL-6 -174. Considering the outcomes observed, we suggest that allele C in IL-6 -147 can escalate the risk of RPL both directly and indirectly, with indirect impact being through the increase in CMV infection. The latter, in turn, might exert significant influence in the occurrence of RPL in women.

CONCLUSION

The evidence implies that the rs1800795 polymorphism of the IL-6 gene may exert a noteworthy impact on the vulnerability to RM in the examined female cohort. Nevertheless, to comprehensively comprehend the effect of this polymorphism on recurrent miscarriage, additional research must be conducted across different populations and regions. Therefore, it is critical to expand the scope of these investigations to enhance our understanding of this genetic factor's contribution to RM.

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Conflicts of interest

There are no conflicts of interest.

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