Short Communication

Study of Cyclophilin A Polymorphism in Women Patients with Cytomegalovirus Infections

Hawraa Ahmed Ali, Mohammed Sabri Abdul-Razzag[1], Milal Muhammad Al-Jeborry[2]

Department of Pharmacy Techniques, Babylon Technical Institute, University Al-Furat Al-Awsat, Najaf, [1]Department of Microbiology, [2]Department of Surgery, College of Medicine, University of Babylon, Hillah, Iraq

Abstract

Background: Cyclophilin A (CypA) is a highly conserved protein that is expressed in a wide range of tissues and cell types and plays roles in inflammation, oxidative stress, and viral infection. CypA may influence oxidative stress pathways during cytomegalovirus (CMV) infection through its modulation of cellular redox balance and signaling pathways involved in oxidative stress responses; CypA is a promising marker of abortion in women infected with CMV. **Aims:** To investigate CypA genetic polymorphism and its serum in Iraqi women with abortion due to infection with CMV. **Methods and Materials:** A total of 200 blood samples were collected from women who had abortions and were admitted to the Maternity and Children's Hospital in Hilla. In total, 50 healthy women control were diagnosed with VIDAS machine for IgM CMV and IgG CMV kits, single nucleotide polymorphism sites of rs4720485 and rs8177826 were analyzed by PCR-RFLP, and CypA serum level determined by enzyme-linked Immunosorbent assay (ELISA). **Results:** Among the total number of samples, we found 60 (30%) CMV were positively obtained from women with abortion, divided into six (3%) samples that were seropositive to CMV IgM, 4(2%) samples that were seropositive to CMV IgM + IgG and 50 (25%) samples positive to IgG only, ELISA analysis showed CypA level concertation with CMV were IgM and IgG are high, analysis for *PPIA* polymorphisms (rs 4720485A/T and rs 8177826C/G) that both are not significant between the patient group and control group. **Conclusions:** To evaluate the role of CypA levels in women who experienced abortion due to CMV infections during pregnancy. The results of the study demonstrated a significant increase in CypA levels in women who were infected with CMV, leading to abortion.

Keywords: CMV, cyclophilin A, polymorphism *PPIA*

Introduction

Cytomegalovirus (CMV) is a DNA virus that has an envelope around it. The virus enters human cells by membrane fusion and can transmit by different routes of transmission, and can manipulate the cellular functions of the host's cells to get the opportunity to multiply and establish a latent infection.[1] When CMV enters the cell, it stimulates the secretion of pro-inflammatory cytokines such as Cyclophilin A (CyPA). During lytic infection, CypA is involved in the production of virus particles, where it can interact with viral proteins such as viral kinases and modulation kinases functions.[2] Moreover, CypA may have a role in latent infection where cyclophilin is required for the establishment of human CMV latency and is associated with the maintenance of the latent viral genome.[3] CypA is a cellular protein found in abundance intra- and extracellularly, intracellular and is involved in protein folding, protein trafficking, and protein activation.[4] The extracellular CyPA is secreted by many types of cells, such as vascular smooth muscle cells, endothelial cells, and macrophages, as a response to oxidative stress; CypA may influence oxidative stress pathways during CMV infection through its modulation of cellular redox balance and signaling pathways involved in oxidative stress responses.[5] A successful microbial infection depends on the host protein CypA. These

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_755_23

Address for correspondence: Dr. Hawraa Ahmed Ali, Department of Pharmacy Techniques, Babylon Technical Institute, University Al-Furat Al-Awsat, Najaf 51001, Iraq.
E-mail: hawraa.ahmed2310@gmail.com

Submission: 13-Jun-2023 **Accepted:** 11-Aug-2023 **Published:** 30-Apr-2026

How to cite this article: Ali HA, Abdul-Razzag MS, Al-Jeborry MM. Study of cyclophilin A polymorphism in women patients with cytomegalovirus infections. Med J Babylon 2026;23:743-7.

proteins may either promote or inhibit virus replication, depending on the host cell type and the viral species. CypA plays an essential role in regulating the life cycle of several etiological agents.[6] CypA is encoded by the peptidyl-prolyl isomerase A (*PPIA*) gene. Human *PPIA* is a 165 amino acid protein conserved across species.[7] The CypA gene is located on human chromosome 7 (7p13) and consists of several exons and introns. The exons are the coding regions of a gene that contain the information necessary for the synthesis of a functional protein. They are transcribed into RNA and then translated into protein. In contrast, introns are noncoding regions of the gene that are transcribed into RNA but are spliced out during the RNA processing steps before translation, while the introns do not contribute directly to the protein-coding sequence. It spans approximately 4.7 kilobases in length.[8] Many diseases may be caused by this virus, but the most common syndrome caused by this virus is its effectivity on a pregnant woman and causes abortion. Several contradictory studies that describe the relationship between human CMV infection and pregnancy loss demonstrate that HCMV can cause miscarriage or stillbirth. Pregnancy-related HCMV susceptibility rates are well documented and among women of childbearing age.[9]

Material and Methods

Study design and blood collection

In the study conducted at the Maternity and Children's Hospital in Hilla governorate, 200 women patients with a history of abortion were examined over a period of 3 months, specifically from March 2022 to May 2022. Additionally, a control group consisting of 50 women with no previous CMV infections was included for comparison. To collect the blood samples, strict aseptic conditions were maintained. Approximately 5 mL of blood was collected from each participant through vein puncture after the skin was properly cleaned. After collection, 2 mL of which were kept in an EDTA tube and used for extraction of human DNA. And 3 mL from the blood samples were allowed to clot by incubating them. Subsequently, the samples were centrifuged at 3000 rpm for a duration of 5 min. This centrifugation process separated the serum from other blood components, and it was then carefully collected in sterile containers. The serum was collected in sterile containers and stored at (-20°C) until tested.

Detection of CMV IgM and IgG

Serological detection for CMV IgM, IgG[10]. All serum samples have estimated the concentration of levels for virus-specific antibodies Anti-IgG and IgM markers using Vidus Kits for CMV IgM, IgG was used (Biomereux Company, Etoile, France).

Measurement of CypA serum level by enzyme-linked immunosorbent assay (ELISA) Kit (E0672Hu)

The level of serum CypA was measured using an ELISA according to the manufacturer's instructions. Before carrying out the experiments, serum samples were brought to room temperature, vortexed, and centrifuged. Absorbance measurements were done in a microplate reader at 450. All samples were run by duplicate, and the concentrations were calculated using a standard curve. The range of determination was 0.5–200 ng/mL for CypA. Serum levels below the lower determination limit were undetectable and considered 0 pg/mL for statistical analysis. For intra and inter-sandwich assays, coefficients of variation of the ELISA kits were <10%.

DNA extraction

Blood samples were collected from the subjects into disposable blood collection tubes (containing anticoagulant EDTA-Na2), and then the samples were immediately transferred to cryopreservation tubes and stored at −80°C. Genomic DNA was extracted and purified from whole blood samples using a genomic DNA purification kit according to the manufacturer's instructions (Geneaid Biotech, Bioneer, Korea). By following these steps, we can obtain high-quality genomic DNA from the blood samples, which can be used.

Genotyping of single nucleotide polymorphisms (SNPs)

Genotyping was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) assay.[11] PCR-RFLP was carried out with 35 cycles of denaturing at 94.8°C for 30 s, annealing at 60.8°C for 30 s, and extension at 72.8°C for 45 s. The PCR product was digested with respective restriction enzymes (New England Biolabs, http://www.neb.com) overnight and then separated into 3% agarose gels. Genotyping primers and conditions are presented in Table 1.

Table 1: Genotyping methods for *PPIA* SNPs

Primer name	PCR primer sequence	Restriction enzyme	Amplicon size	Product size after digestion (bp)
PPIA-489-Forward	cggctggaatgcagtgat	TaqI	199 bp	A: 41,158
PPIA-489-Reverse	acgcttcatcttaggagtacgac			T: 41,55,103
PPIA-1575-Forward	aagtcgcagacccgattg	HaeIII	250 bp	C: 110, 40, 36, 28, 19
PPIA-1575-Reverse	actttctgggccccattc			G: 138, 40, 36,19

Table 2: Comparison of the allele and genotype frequencies between the abortion patient group and control group

SNPs	Allele/Genotype	Abortion group (number, %)	Control group (number, %)	Odds ratio	95% CI	*P* value
rs4720485	**Allele**					
	A	68 (57)	58 (58)	0.947	0.55–1.62	
	T	52 (43)	42 (42)	1.056	0.62–1.81	0.84
	AA	12 (20)	10 (20)	1 (Reference)		
	AT	44 (73.3)	38 (76)	0.96	0.38–2.48	0.82
rs8177826	TT	4 (6.7)	2 (4)	1.67	0.25–11.07	0.82
	Allele					
	C	108 (90)	88 (88)	1.23	0.53–2.87	
	G	12 (10)	12 (12)	0.815	0.35–1.9	0.64
	CC	53 (16.6)	41(8)	1 (Reference)		
	CG	2 (10)	6 (6)	0.26	0.05–1.34	0.2
	GG	5 (75.4)	3 (86)	0.361.29	0.29–5.71	0.2

Ethical approval

The necessary ethical approval was obtained through verbal consent. This study was approved by the Committee of Publication Ethics at the College of Medicine, Babylon Province, Iraq, under reference no, BMS/0203/016.

Results

Human CMV seroprevalences in women with abortion

The prevalence rate of human CMV (HCMV) among women who experienced abortion in a specific population. Among 200 clinical samples from female patients who had one or more abortions and attended the maternity and Children's Hospital in Hilla government over three months from March 2022 to May 2022 of the total number of samples, 60 (30%) samples were positive for the CMV, divided into six (3%) samples were seropositive to CMV IgM, 4 (2%) samples were seropositive to CMV IgM and IgG and 50 (25%) samples positive to IgG only and 140 (70%) of the samples yielding seropositive results for other causes. The control group (50 women) in this study showed negative results for both IgG and IgM, which means the samples were non-infected with this virus.

PPIA genotypes and allele frequencies

Genotyped a total of 110 DNA samples in this study, including 60 patient groups and 50 control groups. The genotypic distributions of SNPs rs4720485 and rs8177826 conformed to HWE in the patient group and control group ($P > 0.05$), as shown in Table 2. The result shows SNPs were found in rs4720485 genotype included A and T where the allele frequency for A (57%) and T (43%) from in women patients with abortion, whereas, in the control group that found the A allele about (58%) and T (42%), this result approve that there are no significant between the patient group and control group in this locus [Figure 1].

Another study on SNPs *PPIA* is rs8177826, included genotypes C and G where the allele frequency for C (90%) and G (10%) from women patients abortion and in a control group that found the C allele (88%) and G allele (12), this result showed there no significant between the patient group and control group in this genetic locus [Figure 2].

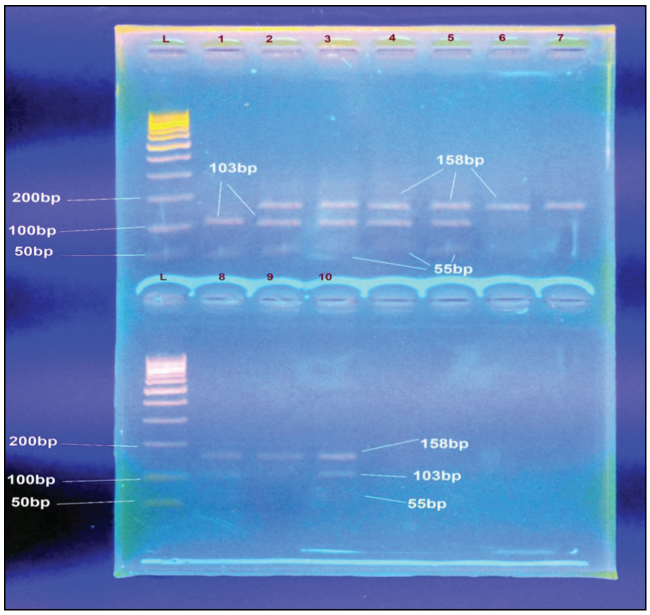

Figure 1: Genotyping of rs4720485 by PCR-RFLP, PCR amplicon 199bp was digested by Taq1 restriction enzyme, which produces 41 + 55 + 103 fragments when T allele present and produce 158 and 41 when A allele present, lanes L = 50 + 100bp step DNA ladder; lanes 5,7 and 9 = AA genotype; lanes 2, 3, 4, 5, 8, and 10 AT genotype; lanes 1 = TT genotype.

Serum levels of CypA in women with abortion

CypA is determined by ELISA, as shown in Table 3; the result showed that the infection with CMV was IgM is high in patient serum, giving rise to an increase in CypA level at high estimate also; CypA levels are also found to be high in women patient with CMV were IgG is high also, This is the first study indicate the relationship between CMV IgM, IgG levels with CypA level, were the previous

Table 3: Correlation analysis between IgM, IgG, and serum CypA level

		CYPA	IGM	IGG	Mean
CYPA	Pearson correlation	1	0.851**	0.801**	26.0730
	Sig. (2-tailed)		0.000	0.000	
	N	110	110	110	
IGM	Pearson correlation	0.851**	1	0.544**	0.7475
	Sig. (2-tailed)	0.000		0.000	
	N	110	110	110	
IGG	Pearson correlation	0.801**	0.544**	1	20.7992
	Sig. (2-tailed)	0.000	0.000		
	N	110	110	110	

**. Correlation is significant at the 0.01 level (2-tailed)

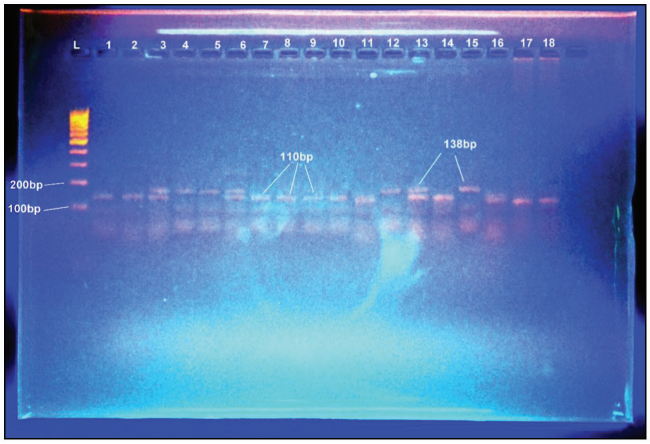

Figure 2: GENOTYPING OF rs8177826 by PCR-RFLP, PCR amplicon 250 bp was digested by HaeIII restriction enzyme, which produces 36 + 47 + 19 + 28 + 110 fragments when the C allele present and produce 36 + 47 + 19 + 138 when the G allele is present, lane L 100 bp DNA ladder; lanes 1, 2, 7, 8, 9, 10, 11, 14, 16, 17, and 18 CC genotype; lanes 3, 6, and 13 CG genotype; lanes 4, 5, 12, and 15 GG genotype.

studies have focused in the relationship between CypA and the virus assembly replication (Keyes *et al*. 2012).

Discussion

Many years ago, CMV was well established to be one of the most important cases of abortion in pregnant women, and till now, this problem has not been solved radically because the virus is considered a hidden virus that when the woman is infected with this virus, the women become symptoms. So, the primary CMV infection in healthy women is mostly subclinical or may be present with mild symptoms.[12] In this study, the prevalence of HCMV infection was 60 (30%) of 200 samples among Iraqi pregnant women. This result is similar to that reported in other research findings (32.78%).[13] But disagree with the lower prevalence in the previous investigation reported in Iraq 56%.[14] This signifies that the immune system begins to produce immunoglobulins in response to the virus. IgG antibody testing is a common method used to screen for and diagnose CMV infections. It detects the presence of CMV-specific IgG antibodies in the blood, indicating prior exposure to the virus. A positive result suggests past infection and the presence of protective antibodies, while a negative result indicates no prior CMV infection or insufficient time for antibodies to develop. IgG testing alone cannot determine the timing or activity of the infection.[15] IgM antibody testing is used to detect recent or acute CMV infections. It helps identify the presence of CMV-specific IgM antibodies in the blood, which are produced by the immune system in response to a new infection. A positive result indicates an active or recent CMV infection, while a negative result suggests either no recent infection or declining antibody levels.[11] When CMV enters the cell, it stimulates the secretion of pro-inflammatory cytokines such as CyPA; CyPA has been found to play a role in HCMV infection, and it can establish both lytic and latent infections in host cells. [16] Our result found that the concertation of serum CypA increased in women abortion with CMV compared to control women healthy ($P < 0.001$). These result is similar to the Sun *et al*.[14] that showed CypA serum levels were significantly higher in severe preeclampsia. Furthermore, they found that CypA was higher in diabetic patients with or without coronary artery disease.[17] This protein is encoded by the CypA gene. CypA is a highly conserved protein that is expressed in a wide range of tissues and cell types. It is known to interact with numerous cellular proteins, including those involved in immune responses, inflammation, and viral infections.[16] The CypA gene, also known as *PPIA* (peptidylprolyl isomerase A), encodes the CypA protein.[18] The result of this study showed that SNP *PPIA* in women abortion for two SNP (rs4720485 and rs8177826) was not significant for genotypic distributions of SNPs in the patient group and control group ($P > 0.05$). This result may be due to a sample scan number that can produce statistical power. On the other hand, this result with the study partially[17] agrees the study polymorphism rs4720485 showed that rs10951772 A/G and rs4720485 A/T PPIA polymorphisms were not significantly associated with the risk of Kawasaki disease development and coronary artery lesion formation, Anthor study conducted on that disagree with our result[19] further provided evidence to indicate that rs8177826

affects transcription of the PPIA gene and transfection with transcription start site -11G mutant promoter could increase in vitro luciferase activities for cells. On the other hand, our result disagrees with An *et al.*[11] whose study was associated with CypA polymorphism rs8177826 in samples, they concluded that CypA significant disease progression infectivity with HIV-1 was reported as the potential genetic modifier.

Conclusion

To evaluate the role of CypA levels in women who experienced abortion due to CMV infections during pregnancy. The results of the study demonstrated a significant increase in CypA levels in women who were infected with CMV, leading to abortion.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Adane T, Getawa S. Cytomegalovirus seroprevalence among blood donors: A systematic review and meta-analysis. J Int Med Res 2021;49:3000605211034656.
2. Wang YQ, Zhao XY. Human cytomegalovirus primary infection and reactivation: Insights from virion-carried molecules. Front Microbiol 2020;11:1511.
3. Abdullah A A, Abdullah R, Nazariah Z A, Balakrishnan K N, Firdaus J Abdullah F, Bala J A, *et al*. Cyclophilin A is a target in the treatment of cytomegalovirus infections. Antivir Chem Chemother 2018;26:2040206618811413.
4. Xue C, Sowden M, Berk BC. Extracellular cyclophilin A, especially acetylated, causes pulmonary hypertension by stimulating endothelial apoptosis, redox stress, and inflammation. Arterioscler Thromb Vasc Biol 2017;37:1138-46.
5. Favretto F, Flores D, Baker JD, Strohäker T, Andreas LB, Blair LJ, *et al*. Catalysis of proline isomerization and molecular chaperone activity in a tug-of-war. Nat Commun 2020;11: 6046.
6. Buxmann H, Hamprecht K, Meyer-Wittkopf M, Friese K. Primary human cytomegalovirus (HCMV) infection in pregnancy. Deutsches Ärzteblatt Int 2017;114:45-52.
7. Ghailan SH, Mohammed SS. Assessing the risk factors for cytomegalovirus and prediction the relationship between abortion and virus in Kirkuk city hospitals. Mosul J Nurs 2020;8:127-41.
8. Khudhair SA, Al-Azzawi RH. Estimation of anti-CMV antibodies in Iraqi pregnant women infected with chronic cytomegalovirus. J Glob Pharma Technol 2018;10:52-6.
9. Shaker Jaber M, Shubber HW, Mohammed GJ. Molecular and immunological detection of *Toxoplasma gondii* and cytomegalovirus in aborted women with COVID-19. J Surv Fish Sci 2023;10:4383-402.
10. Al-janabi HS, Kelkawi AH, Abdul-Hsin IF, Kadhim MJ, Hussein MM. Comparative evaluation study of ELISA system and MINI-VIDAS system for detection of cytomegalovirus IgM antibodies. J Pharm Sci Res 2018;10:2549-50.
11. An P, Wang LH, Hutcheson-Dilks H, Nelson G, Dronfield S, Goedert JJ, *et al*. Regulatory polymorphisms in the cyclophilin A gene, PPIA, accelerate the progression to AIDS. PLoS Pathog 2007;3:e88.
12. Onpoaree N, Sanpavat A, Sintusek P. Cytomegalovirus infection in liver-transplanted children. World J Hepatol 2022;14:338-53.
13. Liao Y, Luo D, Peng K, Zeng Y. Cyclophilin A: A key player for etiological agent infection. Appl Microbiol Biotechnol 2021;105:1365-77.
14. Sun W, Xu Y, Xin Q, Zhang Y, Cui B, Hong F. Association between polymorphism in cyclophilin A gene and its serum and placental expression in Han Chinese women with severe preeclampsia. Pregnancy Hypertens 2019;15:84-92.
15. Ramachandran S, Venugopal A, Kutty VA, Chitrasree VA, Mullassari A, Pratapchandran NS, *et al*. The plasma level of cyclophilin A is increased in patients with type 2 diabetes mellitus and suggests the presence of vascular disease. Cardiovasc Diabetol 2014;13:1-8.
16. Zhou QQ, Wang Y, Hu JJ, Zhang L, Li JB, Xu YJ, *et al*. Identification and characterization of a cyclophilin A gene from Chinese shrimp *Fenneropenaeus chinensis*: Sequence features and expression profiles. Invertebr Surv J 2022:105-14.
17. Shi R, Luo Y, Li S, Kong M, Liu X, Yu M, *et al*. Single-nucleotide polymorphism rs17860041 A/C in the promoter of the PPIA gene is associated with susceptibility to Kawasaki disease in Chinese children. Immunol Invest 2021;50:230-42.
18. Hadpech S, Thongboonkerd V. Current update on theranostic roles of cyclophilin A in kidney diseases. Theranostics 2022;12:4067-80.
19. Palacin M, Rodriguez I, García-Castro M, Ortega F, Reguero JR, López-Larrea C, *et al*. A search for cyclophilin-A gene (PPIA) variation and its contribution to the risk of atherosclerosis and myocardial infarction. Int J Immunogenet 2008;35:159-64.