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A Serology and Immunological Markers to Assess the Relationship between CMV and UTIs in Miscarriage

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Abstract

Cytomegalovirus (CMV) is a prevalent virus that can play a role in urinary tract infections (UTIs) among women, especially during pregnancy. The research seeks to establish the prevalence of Cytomegalovirus infection and evaluate particular immunological markers such as Interleukin-10 (IL-10), Interferon-γ (IFNγ), and Procalcitonin (PCT) in pregnant and non-pregnant women with UTIs.

This case-control study, based on data, included 200 women with possible UTIs. Participants were categorized into four study groups according to their pregnancy and miscarriage experience: Group I comprised pregnant women with a past miscarriage history; Group II included pregnant women with no prior miscarriage history; Group III consisted of non-pregnant women with a history of miscarriage; and Group IV represented non-pregnant women without a history of miscarriage. Serum samples were analyzed for IL-10, IFNγ, PCT, and Immunoglobulin M (IgM) and immunoglobulin G (IgG) to CMV using Enzyme-Linked Immunosorbent Assay.

From 200 blood samples collected from women with UTIs. IgM antibodies to CMV were detected ($p<0.001$), indicating an acute infection. IgG levels exhibited substantial differences between G1 and G2, being highest in G1, moderate in G2, and lowest in women without a history of miscarriage in G3 and G4. The findings showed marked significant variations in PCT, IL-10, and IFN-γ among the study groups, $p < 0.001$.

The study shows a notable prevalence of UTIs in women and suggests a potential link between CMV-seropositivity and miscarriage. The findings emphasize the significance of bacterial and viral infections in women with UTIs, especially during pregnancy.

Keywords: Cytomegalovirus (CMV), Urinary tract infections (UTIs), Miscarriage, Immunological markers.

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Introduction

Cytomegalovirus (CMV) is a common herpesvirus worldwide (1,2) and is categorized as a β-herpesvirus (HHV-5). Approximately 60–70% of the worldwide population harbors cytomegalovirus, which usually stays dormant, especially in healthy people (3). Transmission occurs via direct contact with bodily fluids, including blood, semen, vaginal secretions, saliva, tears, urine, feces, and breast milk (4). Pregnant women often contract cytomegalovirus through sexual relations or frequent interactions, especially with young children in daycare (5). The majority of congenital CMV instances and associated disabilities arise from primary infections occurring during pregnancy (6).

Urinary tract infections (UTIs) occur due to bacterial entry into the urinary tract (UT) (7). Women experience the greatest impact from UTIs because of anatomical characteristics that promote bacterial colonization in the female urogenital system. Half of all women will have at least one UTI during their lifetime (8). During pregnancy, the microorganisms causing UTIs are similar to those in non-pregnant individuals, with *E. coli* responsible for 70-80% of cases (9,10). Globally, the prevalence of symptomatic UTI in pregnant women is between 4% and 47%, while asymptomatic bacteriuria varies from 2% to 10%. In Iraq, the prevalence of UTIs among pregnant women has been recorded at about 47% in Mosul and 65% in Babil (11). Physiological and anatomical changes during pregnancy, as well as alterations in the immune system, heighten the chances of asymptomatic bacteriuria, which can occasionally progress to symptomatic infections, presenting significant dangers to both mother and fetus. Attributes like age, numerous pregnancies, diabetes, sickle cell anemia, past UTI occurrences, urinary tract disorders, and immunodeficiencies can increase the likelihood of UTI during pregnancy (12). This study aims to assess whether CMV is involved in UTIs in female patients, both pregnant and non-pregnant. It develops an extensive diagnostic approach by evaluating various serological and immunological markers, estimating CMV-specific IgG and IgM levels through ELISA to determine infection status. The research additionally examines essential pro-inflammatory cytokines like Interleukin-10 (IL-10), interferon gamma (IFN-γ), and Procalcitonin (PCT), aiming to comprehend their functions in the urinary tract and their effects on women's health.

Material and Methods:

Study design and setting:

This observational case-control research took place at three hospitals in Baghdad from August to November 2025. The research group consisted of pregnant and non-pregnant females between the ages of 13 and 45 years.
A total of 200 participants were included in the study. A total of 200 patients had UTIs. Essential demographic and clinical data were collected at baseline for each participant. Patients receiving antibiotics, chemotherapy, radiotherapy, or immunosuppressive treatment were not included.

Participants were classified into four groups based on their pregnancy and miscarriage status. Group I consisted of pregnant women with a history of miscarriage, Group II included pregnant women without a history of miscarriage, Group III was made up of non-pregnant women who had a history of miscarriage, and Group IV represented non-pregnant women without a history

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of miscarriage. Every group consisted of 50 participants.

Sample Collection:

Blood samples were obtained from all participants at baseline to evaluate serological markers, including IL-10, IFN-γ, and PCT. The samples were housed in gel tubes, centrifuged according to standard procedures for biological specimens, and kept at 4°C before testing. Serum was subsequently examined for IgM and IgG antibodies against CMV employing enzyme-linked immunosorbent assay (ELISA).
Serum samples were isolated through centrifugation and preserved according to standard laboratory protocols until analyzed. The immunological markers IL-10, IFN-γ, PCT, and CMV-specific IgG and IgM were measured through sandwich ELISA. Assays for IL-10, IFN-γ, and PCT adhered to the manufacturer's guidelines, utilizing commercial ELISA kits from Elabscience (China). In contrast, IgG and IgM were measured using kits from OriGene (USA).

Statistical analysis: Statistical analysis entails complex computations of ratios and performing chi-square tests to evaluate the significance (P-value) among the examined factors. One-way ANOVA compared the various groups, with results presented as mean ± standard deviation (SD). The LSD test revealed significant variations among means, with letters (A, B, and C) indicating levels of significance—A denoting extremely significant and decreasing thereafter. A $p \leq 0.05$ indicated possible significance. The evaluation of contingency tables was performed with SPSS (v 20).

Results:

Levels of CMV IgM and IgG antibodies are evaluated in all investigated groups. A single woman, who had an early miscarriage recently, tested positive for IgM antibodies and displayed severe infection symptoms, with elevated IgM antibody levels as indicated in Table 1.
The CMV IgG levels differed across the groups, with positive cases exhibiting varying post-recovery levels, indicating prior infection. The analysis revealed statistically significant differences, with the least difference observed in Table 2. G1 exhibited peak levels of IgG.

Regarding procalcitonin (PCT) concentrations among the groups, Table 3. The G1 showed the greatest average PCT concentration, with G3 next. These values surpass the standard limit of below 0.5. Both groups comprised female participants with a history of abortions, who mainly faced UTIs.

Table 1: Comparative-statistical-analysis-of-concentration of IgM across all tested groups.

IgM IU/mL Normal value ≤ 0.99	N	Minimum	Maximum	Mean	Std. Deviation	P-value
G1	50	0.00	38.92	**1.03**	0.43	0.05
G2	50	0.00	0.18	**0.02**	0.04	
G3	50	0.05	0.01	**0.01**	0.02	
G4	50	0.05	0.03	**0.001**	0.01	

GI: pregnant women with a history of miscarriage, GII: included pregnant women without a history of miscarriage, GIII: non-pregnant women who had a history of miscarriage, and GIV: represented non-pregnant women without a history of miscarriage

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Table 2: Comparative statistical analysis of the concentration of immunoglobulin G antibody across all tested groups.

IgG IU/mL Normal value ≤ 0.99	N	Minimum	Maximum	Mean	Std. Deviation	P-value
G1	50	0.05	8.30	7.08	4.48	0.05
G2	50	0.04	0.43	0.25	0.13	
G3	50	0.04	2.06	0.62	0.15	
G4	50	0.03	0.09	0.033	0.16	

GI: pregnant women with a history of miscarriage, GII: included pregnant women without a history of miscarriage, GIII: non-pregnant women who had a history of miscarriage, and GIV: represented non-pregnant women without a history of miscarriage

Table 3: Statistical comparison of Procalcitonin levels across all tested groups

Procalcitonin ng/ml 0.05-0.5 ng/ml	N	Minimum	Maximum	Mean	Std. Deviation	P-value
G1	50	0.83	2.45	**A (1.83)**	0.2	0.001
G2	50	0.09	0.1	**C (0.14)**	0.05	
G3	50	0.21	1.2	**B (1.12)**	0.15	
G4	50	0.05	0.07	**D (0.11)**	0.02	

GI: pregnant women with a history of miscarriage, GII: included pregnant women without a history of miscarriage, GIII: non-pregnant women who had a history of miscarriage, and GIV: represented non-pregnant women without a history of miscarriage

Regarding IFN - γ concentrations among the groups. G1 exhibited the highest levels of IFN-γ among the groups, signifying a markedly heightened inflammatory response, as outlined in Table 4. G2 exhibited notably reduced IFN-γ levels. G3 exhibited moderate levels of IFN-γ, indicating immune activation associated with cytomegalovirus infection.

Table 4: Statistical comparison of IFN–γ levels across all tested groups.

IFN –γ 0-15 pg/ml	N	Minimum	Maximum	Mean	Std. Deviation	P-value
G1	50	57.97	74.23	**A (69.71)**	11.99	0.001
G2	50	9.33	12.22	**C (11.24)**	2.33	
G3	50	30.56	33.07	**B (28.49)**	5.62	
G4	50	4.87	10.66	**D (9.68)**	1.49	

GI: pregnant women with a history of miscarriage, GII: included pregnant women without a history of miscarriage, GIII: non-pregnant women who had a history of miscarriage, and GIV: represented non-pregnant women without a history of miscarriage

Association between cytomegalovirus and UTIs Severity and Concentration of interluken 10

Table 5 indicates that significant differences exist at the 0.05 level among the four groups. Most IL-10 levels remained normal, but many of the G1 patients exhibited levels surpassing the upper limit, indicating a potential inflammatory reaction. The maximum level noted was 10.99, exceeding the usual limit

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Table (5): Statistical comparison of Interleukin-10 across all tested groups.

Interleukin-10 pg/ml 1-9.1 pg/ml	N	Minimum	Maximum	Mean	Std. Deviation	P-value
G1	50	1.47	10.9	**A (8.9)**	2.17	0.05
G2	50	1.10	6.52	**C (2.16)**	1.54	
G3	50	1.70	6.43	**B (3.20)**	1.76	
G4	50	1.32	4.86	**C (1.73)**	0.98	

GI: pregnant women with a history of miscarriage, GII: included pregnant women without a history of miscarriage, GIII: non-pregnant women who had a history of miscarriage, and GIV: represented non-pregnant women without a history of miscarriage

Discussion

The results indicate a strong connection between CMV and UTIs, particularly in women with a history of miscarriage. This research evaluated IgM and IgG antibody levels in four groups to explore the link between CMV infection and miscarriage. Only one pregnant woman with a history of miscarriage tested positive for IgM at the highest level, suggesting a recent CMV infection. Although IgM positivity was rare in the studied group, the significant P-value ($p = 0.001$) suggests that acute CMV infection during pregnancy can result in important clinical consequences.

The analysis revealed significant statistical changes ($P \leq 0.05$) in all assessed groups. These results suggest a likely connection situated between IgG levels and pregnancy complications, highlighting the immune reaction provoked by viral agents such as CMV. G1 showed the highest IgG antibody levels, signifying an immune response activated by infectious agents or immune activation associated with miscarriage (13). G4 exhibited moderate IgG levels and markers of immune activation, indicating possible previous exposure to pathogens or a compromised immune response. In contrast, G2 and G4 showed the lowest levels of IgG, suggesting an immediate immunological threat. IgG and IgM antibodies serve as crucial biomarkers reflecting the connection between CMV occurrence and the intensity of the related disease (14). These antibodies are crucial elements of the inflammatory immune response, involved in neutralization, opsonization, and antibody-dependent cell-mediated cytotoxicity, all of which greatly influence the advancement of both mild and severe disease symptoms (15).

Severe CMV infection, as shown by positive IgM, was infrequent in the study, with just one pregnant woman who had a history of miscarriage affected. Although uncommon, the significantly high IgM levels and the statistical relevance ($P = 0.001$) suggest that early CMV infection during pregnancy could result in severe health complications, including a higher chance of miscarriage. This implies that, despite prior studies indicating that, although infrequent, severe CMV infections can pose a major risk during pregnancy (16).

IgG antibodies against CMV were detected more frequently, solely in women with a history of miscarriages. The cohort of pregnant women with previous miscarriage exhibited the highest IgG levels, suggesting prior exposure or current infection. Elevated IgG levels may indicate activation of the immune response or viral

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reactivation, potentially contributing to pregnancy complications through immune-related mechanisms (17).

A significant association was identified between CMV infection and UTIs in women who had miscarriage. In the group with miscarriage during pregnancy, every CMV-positive person also tested positive for urine cultures, and this group showed the highest rates of CMV infections and UTIs (P >0.001). Comparable results were observed in non-pregnant women who underwent miscarriage, suggesting that immune changes induced by CMV might increase the likelihood of bacterial UTIs, irrespective of pregnancy (18).

Interleukin-10 is a cytokine that diminishes inflammation and affects immune reactions (19). It is generated by different immune cells, such as regulatory T cells, monocytes, and B cells (20). Its primary functions involve inhibiting pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, IL-6, and IFN-γ; reducing the expression of MHC class I and II; and regulating immune signaling suppressors (19). Interleukin-10 plays a crucial role in maintaining the equilibrium between antimicrobial defense and tissue protection (21). In a UTI, its concentrations rise in blood and urine as part of an anti-inflammatory mechanism, intended to minimize tissue injury and manage inflammation (22).

A comprehensive analysis revealed that people suffering from UTIs showed significantly elevated levels of Interleukin-10 when compared to control subjects, suggesting that anti-inflammatory immune responses could influence the body's response to bacterial invaders (23). Studies show that increased urinary Interleukin-10 levels are associated with persistent bacterial colonization and may elevate the chances of repeated infections (24).

Cytomegalovirus produces a viral version of Interleukin-10 (CMVIL-10, ORF UL111A). This viral variant engages with IL-10 receptors on human cells, consequently suppressing immune responses by significantly lowering inflammatory cytokine production and the activity of inflammatory cells (25). Consequently, the virus not only exploits the anti-inflammatory characteristics of IL-10 but also employs this viral variant to escape immune detection, resulting in noticeable effects like suppression of T cell activation, lowered inflammatory cytokine levels, and impairment of major histocompatibility complex (MHC) functionality (26). During CMV infection, human Interleukin-10 is produced to reduce inflammation and limit tissue damage; however, it also promotes viral persistence by dampening the immune response, especially in the initial or moderate stages of infection (17).

IFN-γ is mainly generated by Th1 and NK cells, which serve as vital mediators in the induction of antiviral immune responses (27). An abnormal rise in IFN-γ levels might disturb the immune balance required for maintaining pregnancy, which could clarify the link between heightened IFN-γ levels and the incidence of miscarriage (28).

The lowest amounts of IFN-γ were noted in the fourth cohort (non-pregnant individuals without a history of miscarriage), signifying limited immune activation. This finding aligns with the lack of viral pathogenicity and gestation, signifying a stable immunological condition (29).

Procalcitonin (PCT) is a biomolecule that serves as a precursor to calcitonin and is produced in minimal quantities under normal physiological

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conditions, usually below 0.05 ng/ml (30). Throughout gestation, increased levels of PCT might signify an uncontrolled inflammatory reaction that threatens the stability of the pregnancy (31). The peak levels of PCT noted in different cohorts indicate considerable bacterial inflammation, increased activation of the innate immune response, and the possibility of developing ascending UTIs or secondary bacterial infections linked to CMV. An overproduction of inflammatory cytokines may lead to placental impairment and disrupt immune balance during pregnancy, characterized by Th1 dominance. This could also result in abortion (32).

This research emphasizes procalcitonin as a crucial immune indicator for evaluating the severity of bacterial infections. Elevated procalcitonin levels are strongly associated with abortion in both pregnant and non-pregnant populations. These results bolster the notion that inflammatory reactions play a role in the initiation of threatened abortion (31).

Conclusion

This study indicates a notable connection between CMV infection and UTIs, particularly in women who have a history of previous miscarriage. Elevated levels of IgG and IgM antibodies indicate previous exposure, viral reactivation, or infection during pregnancy, which may negatively impact pregnancy outcomes. Immunological testing shows that IL-10 serves as an important anti-inflammatory agent, while IL-10 produced by CMV can suppress immune responses, enabling the virus to survive. Elevated amounts of pro-inflammatory cytokines and procalcitonin (PCT) indicate bacterial inflammation and activation of the innate immune response, potentially affecting immune stability during pregnancy. Monitoring immune markers may help in predicting and controlling infection risks during pregnancy.

Ethical considerations:

Prior to the start of the study, informed consent for participation was secured from all members involved (BCSMU/80025/00079 at August 1, 2025).

Author contributions

All authors contributed to the collection of data and the preparation of the draft, and read and approved the final version of the manuscript.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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